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Saudi Scientific Diabetes Society Position Statement: Management of Diabetes Mellitus in the Pandemic of COVID-19

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Abstract

About 10% coronavirus (COVID-19) infected patients are with diabetes comorbidity. Also, diabetes promotes severe progression in COVID-19 patients. Diabetes comorbidity is associated with significant mortality in those people with COVID-19. In this position statement, the management of diabetes in cases of COVID-19, has been presented. The impact of diabetes on the morbidity and mortality of COVID-19, as well as both the target glucose level and the method of blood glucose control have been presented in details.

Keywords

COVID-19, Diabetes Mellitus, Glycemic Target, Blood Glucose Control

1. Introduction

During our current era, the pandemic of coronavirus disease 2019 (COVID-19) has drawn the attention of every one all over the globe. In April 6th 2020, globally, according to the World Health Organization (WHO), CoVID-19 affected 1,210,956 confirmed cases with 67,594 deaths, with its unprecedented challenges to the global public health system [1]. This pandemic has disrupted the flow of everyday life as we know it. Healthcare systems started to experience some failure which led to reallocation of its resource in a new setting because of this crisis with a great difficulties for both healthcare personals and patients. Of course, people with comorbid diseases like diabetes mellitus are at a great risk for the hazardous consequences of this pandemic. Thus, the Saudi Society of Diabetes

would like to provide its recommendations for the clinical care of diabetes during this COVID-19 pandemic.

2. Diabetes Mellitus and COVID-19

2.1. Diabetes Mellitus and COVID-19 Morbidity and Mortality

Diabetes promotes severe progression in COVID-19 patients, the presence of coexisting diabetes was more common among COVID-19 patients with severe disease than among those with non-severe disease (16.2% vs. 5.7%), (**Figure 1**). Data was extracted regarding 1099 patients with laboratory-confirmed Covid-19 from 552 hospitals in 30 provinces, autonomous regions, and municipalities in China through January 29th, 2020 [2]. Another nationwide analysis of comorbidity and its impact on 1,590 patients with COVID-19 in China, also, revealed thatCOVID-19 patients with diabetes had a worse prognosis (**Figure 2**) [3].

Diabetes is considered as a risk factor for mortality in cases with COVID-19. A large national study showed that the mortality of patients with diabetes was significantly higher than that of non-diabetic patients, (10% vs 2.5% P <0.001) [3]. In addition, WHO Joint Mission claimed that patients who reported no comorbid conditions had a CFR of 1.4%, patients with comorbid conditions had

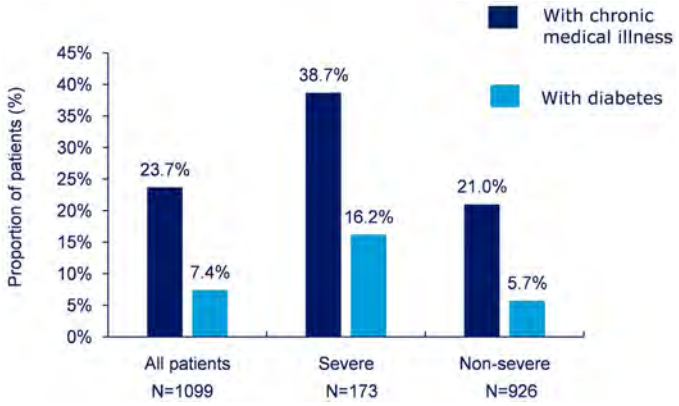

Figure 1. COVID-19 patients with comorbidities [2].

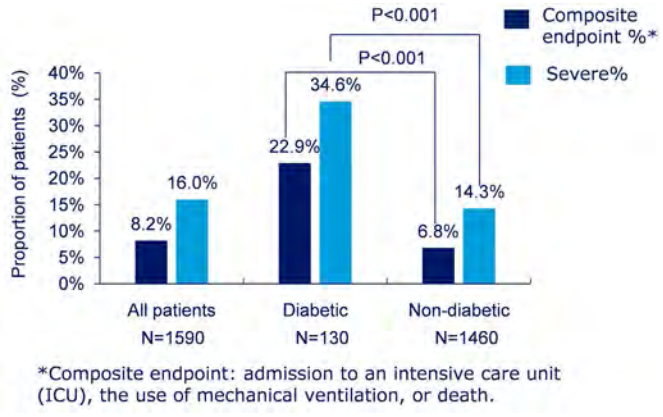

Figure 2. The risk of reaching to the composite endpoints and disease severity among patients with COVID-19 [3].

much higher rates—9.2% for diabetes [4]. Also, Chinese CDC declared that patients who reported no comorbid conditions had a case fatality rate (CFR) of 0.9%, while patients with comorbid conditions had much higher rates—7.3% for diabetes [5]. A research about clinical characteristics of 82 death cases with COVID-19 found that diabetic ketoacidosis is one of the causes of mortality [6].

2.2. How Does Diabetes Accentuate COVID-19 Morbidity?

High level IL-6, TNF-α and other inflammatory cytokines are expressed in the serum of individuals with diabetes [7]. Moreover, animal models suggested that diabetes significantly promoted the production of TLR4-induced IL-6 [8]. Also, studies have shown that coronavirus, including SARS, is highly likely to activate TLR3 and TLR4, leading to a runaway immune response [8], which in turn leads to Il-6-dominated cytokine storms, which have also been shown to be one of the leading causes of death from coronavirus pneumonia [9]. In addition, IL-6 were associated with death of COVID-19 patients, univariate regression showed increasing odds of in-hospital death associated with IL-6 (odds ratio 1.12, 95% CI 1.03 - 1.23, Per 1 unit increase; p = 0.0080) [10]. Therefore, it can be speculated that over activation of TLR4 signaling (**Figure 3**) in individuals with diabetes may contribute to the progression of the severity of the disease and even mortality.

Moreover, patients with diabetes may have bad glycemic control after viral

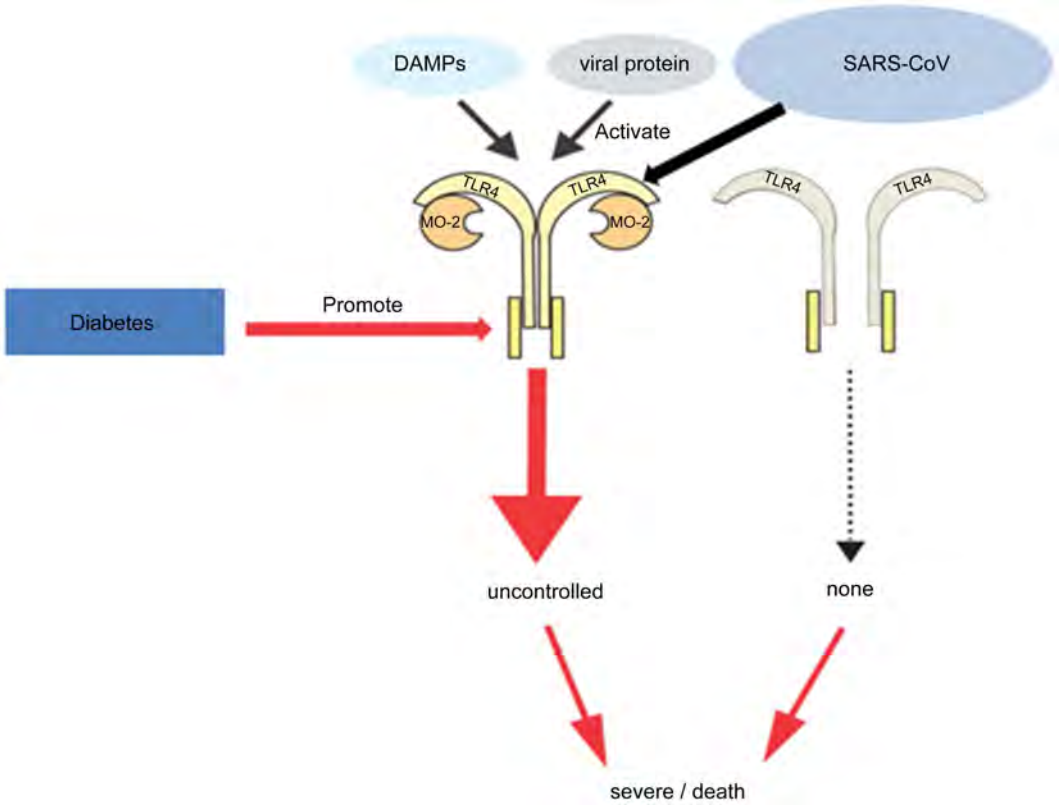

Figure 3. The potential mechanism of high severe/died risk in diabetic patients [10].

infection. It was suggested that SARS-CoV may cause pancreatic islets pathology because of the expression of angiotensin converting enzyme 2 (ACE2), the functional receptor of SARS-CoV, in the exocrine and endocrine tissues of the pancreas [11]. In addition, a recent study in China have found that SARS-CoV-2 is able to efficiently use human ACE2 as a receptor for cellular entry, which could potentially facilitate human-to-human transmission [12].

2.3. Glucose Level Fluctuation in Patients with COVID-19

Glucose level is fluctuating in patients with diabetes and COVID-19 because of irregular diet, reduced exercise, increased glucocorticoids secretion due to stress, the use of glucocorticoids in treatment, the interruption or non-standard treatment with oral antidiabetic drugs (OAD) in isolation wards. Also, COVID-19 can cause human body to produce a large number of inflammatory cytokines and lead to extreme stress in some severe and critical patients [13].

3. Management of Diabetes Mellitus in Patients with COVID-19

3.1. Glycemia Target in Patients with COVID-19

Stratification of cases with COVID-19 according to severity is the first step for better glucose management. For mild and moderate non-elderly COVID-19 patients, stick with strict high control target (Table 1). For mild and moderate elderly patients, or patients who have been using glucocorticoid, set up a low or strict control target. For severe and critical patients, elderly patients, hypoglycemia intolerable patients, or patients who have organ dysfunction or serious cardiovascular and cerebrovascular diseases, set up a low control target (Table 1). Also, hypoglycemia occurrence should be minimized during glucose management in diabetes patients with COVID-19. Medical care should be performed in time if hypoglycemia occurs [13].

3.2. Therapeutic Principle of Glucose Management in Patients with COVID-19

Insulin treatment is the first choice if diabetes combined with severe infection. For non-critical patients, insulin sc. injection is recommended, and basic dosage can refer to the out-of-hospital dosage. For critical patients, CSII is recommended. In case of serious glucose metabolism disorder with water and electrolyte and acid-base disorders, IV insulin treatment should be started, in combination with aggressive fluid infusion. If the clinical condition is stable and eating

Table 1. Target stratification of glucose management in hospitalized patients.

	High	Medium	Low
FPG/PPG, (mmol/L)	4.4 - 6.1	6.1 - 7.8	7.8 - 10.0
2hPPG/GLU, (mmol/L)	6.1 - 7.8	7.8 - 10.0	7.8 - 13.9

pattern is regular, patients can continue OAD treatment as before admission. Using NPH and long-acting insulin is recommended during glucocorticoid treatment to control the glucose level. Also, measure 7 point glucose (if necessary, plus nocturnal glucose) during insulin treatment [13]. Antidiabetic drugs and their use are shown in Table 2.

3.3. Management Plan of Diabetes in Patients with COVID-19 according to Disease Severity

Severity of COVID-19 can be based on the clinical classification released by the National Health Commission of China as shown in Table 3 [14].

1) Mild cases

There is no need to adjust the original regime too much. Both OAD and insulin treatment can be maintained. The progress of COVID-19 can be rapidly,

Table 2. Anti-diabetic drugs and COVID-19.

Class	Comment
Metformin	Not recommended in severe/critical patients; patients with gastrointestinal symptoms or lack of oxygen.
Secretagogue	Mild/moderate patients using glucocorticoid: • Short-acting agents can be used in early stage; • Middle/long-acting agents if FPG and/or PPG increased or in advanced stage.
α-glucosidase inhibitor	Can be used to control PPG. Not recommended in severe/critical patients; or in patients with gastrointestinal symptoms.
TZD	Can be used during the process of glucocorticoid treatment. Regime should be adjusted according to treatment effect.
DPP-4i	Not recommended
SGLT-2i	Not recommended for COVID-19 patients having stress reaction at different levels.

Table 3. Severity classification of COVID-19.

Mild	Mild clinical manifestation None Imaging Performance
Moderate	Fever, respiratory symptoms, pneumonia performance on X-ray or CT
Severe	Meet any of the followings: 1. Respiratory distress, RR ≥ 30/min; 2. Oxygen saturation ≤ 93% at rest state; 3. Arterial partial pressure of oxygen (PaO_2)/Fraction of inspiration O_2 (FiO_2) ≤ 300 mnHg, 1 mmHg = 0.133 kPa
Critically severe	Meet any of the followings: 1. Respiratory failure needs mechanical ventilation; 2. Shock; 3. Combined with other organ failure, patients need ICU monitoring and treatment

and can be worsen with hyperglycemia, thus, it is strongly recommend to increase the frequency of glucose monitoring, and consult with physicians to adjust treatment regime whenever needed even in diabetes patients with mild COVID-19.

2) Moderate cases

Original treatment regime is to be maintainedif patient's cognitive condition, appetite and glucose control are within normal range. In patients with obvious COVID-19 symptoms who cannot eat regularly, OAD is to be switched to insulin. Premix insulin regime is to be switched to basal-bolus regime or insulin pump for better flexibility of glucose management.

3) Severe and critical cases

Intravenous insulin injection should be the first-line treatment. For patients who are in process of continuous renal replacement therapy (CRRT), the proportion of glucose and insulin in the replacement solution should be increased or decreased according to glucose monitoring result to avoid hypoglycemia and severe glucose fluctuations.

3.4. Management Plan of for Different Types of Diabetes in Patients with COVID-19

1) Type 1DM

Insulin pump or basal plus bolus insulin treatment is the optimal regimen. Insulin analogues are recommended as first choice. Insulin treatment regimenis to be individualized according to individual's profile.

2) Type 2DM

For mild COVID-19 patients with low-moderate glucose increase, non-insulin diabetes drugs can be used. For patients with fever or treated by glucocorticoid, insulin treatment is the first choice. For critical patients, insulin IV injection is recommended.

3) Glucocorticoid-associated diabetes

Glucocorticoids-induced glucose increase often occurs between after-lunch and before-sleep. Therefore, it is important to monitor blood glucose after lunch and before dinner. Insulinisthe first choice.

3.5. Glucose Management of COVID-19 and Diabetes Treated with Glucocorticoids

Effect of glucocorticoids in COVID-19 is uncertain. However, some centers reported that glucocorticoids can be used in critical patients, or patients with fever and sporadic lesion in lungs, to reduce inflammatory lung injury. Thus, special consideration should be taken in management of glucose level in those cases [13].

3.6. Glucose Management of Children and Adolescents

In children, strengthen blood glucose monitoring (4 - 7 times/day) and individualize the exercise program. The total energy requirement in kcal/day = (Age *

70 - 100) + 1000 with carbohydrate: 50% - 55%, fat: 25% - 35% and protein: 15% - 20%. It is to be adjusted by nutrition, physical activity and stress of the children. Identify DKA and hypoglycemia in time. Target of blood glucose control is HbA1c < 7.5% [15] [16].

4. Recommendations

- Because of the more fluctuations of blood glucose level, strengthened dynamic glucose monitoring is necessary.
- As disease conditions of COVID-19 and diabetes can change rapidly, it is recommend to apply insulin therapy as soon as possible according to glucose level, and actively adjust treatment regime, to control glucose to a relatively ideal level.
- Insulin IV injection should be the first-line treatment in severe and critical patients.
- For fasting patients, the suggested ratio of glucose to insulin in IV infusion fluid is 2 - 4 G glucose: 1U insulin.
- Too much and too long course of glucocorticoid treatment are main risk factor for glucose deterioration.
- When gradually reduce glucocorticoid treatment, we should also pay attention to glucose fluctuation, and adjust insulin dosage according to glucose level.
- In principle, it is recommended to measure blood glucose 7 times a day.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Research Progress of C-Peptide and Its Physiological Function

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Abstract

As a product in the process of insulin synthesis, C-peptide's physiological function is still not very clear. Recent studies have shown that C-peptide has many potential cell targets and has biological effects on a variety of tissue systems in humans and other animals. In this paper, the effects of C-peptide on diabetic complications, reproductive endocrine system, blood system, tissue repair, and neoplastic diseases were reviewed to provide references for further clarification of c-peptide related problems.

Keywords

C-Peptide, Physiological Functions, Review

1. Introduction

C-peptide is a polypeptide composed of 31 amino acids; it is a key substance connecting proinsulin alpha chain and beta chain, and plays an important role in the correct folding of proinsulin and the formation of disulfide bonds. Endogenous or exogenous substances such as glucose, amino acids, glucagon, etc. stimulate β cells to release equal amounts of insulin and C-peptide from their secretory vesicles. The regulation of insulin on blood glucose, fat and protein metabolism has been very clear, and has long been used clinically as a hypoglycemic drug. As a homologous active substance secreted by insulin, C-peptide has not been paid enough attention for a long time, and its physiological function is still not very clear. C-peptide was previously considered to be inert, but now more and more studies have shown that C-peptide is a physiologically active molecule with many potential cellular targets [1]; it can play an important role in diabetic complications, reproductive endocrine system, blood system, tissue re-

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pair, tumor diseases and other aspects.

2. The Role of C-Peptide in Diabetic Complications

2.1. Effect of C-Peptide on Kidney

Diabetic nephropathy (DN) is a serious diabetic microvascular complication; it is the main cause of end-stage renal disease and the leading cause of death in T1DM patients. The appearance of urinary protein and the gradual decline of renal function are the signs of the development of DN.

Studies have shown that C-peptide has the effect of improving DN, and its protective effect on the kidney is reflected in two aspects of structure and function. A study in rats showed that physiological dose of C-peptide treatment can reduce urinary protein, improve glomerular sclerosis and podocyte morphology, reduce the thickness of glomerular basement membrane, and inhibit the synthesis of glomerular mesangin [2]. Another Meta analysis showed that C-peptide can reduce glomerular hyperfiltration and reduce urine protein in T1DM patients with normal renal function or early renal disease; C-peptide can reduce urine protein, diminish glomerular volume and mesangial matrix area in diabetic animals [3]. However, research by Nakamoto *et al*. showed that C-peptide did not improve the glomerular filtration membrane structure of early T1DM rats, and believed that the protective effect of C-peptide on reducing glomerular filtration rate was functional, not Structural [4]; but the duration of this study is short (only 1 day of C-peptide injection), and its long-term effect is unknown, so the conclusion is open to question. Evaluation of the effect of pancreatic transplantation surgery further supports the beneficial effect of C-peptide on DN, because renal function in patients with T1DM can be partially improved after transplantation, which is considered to be due to the supplementation of C-peptide [5].

Research by Li *et al*. showed that C-peptide hardly enters glomerular cells when blood glucose levels are normal, but C-peptide can be dynamically localized in the nucleus after high glucose stimulation, which provides a basis for elucidating the mechanism of C-peptide on DN [6]. The protective mechanism of C-peptide on DN is as follows: a) C-peptide stimulates eNOS gene transcription, inhibits iNOS expression, down-regulates RAGE and up-regulates PKA, and prevents and delays microangiopathy in the kidney [2] [6]. b) Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases, which can degrade many types of extracellular matrix components.C-peptides may reduce the accumulation of DN extracellular matrix by delaying MMP-9 expression, thus reversing DN [7]. c) C-peptide may be used as a natural antioxidant to protect the function of islet β cells and delay DN [8]. d) C-peptide can play a protective role in the kidney by reducing the inflammatory response [9].

2.2. Effect of C-Peptide on Retina

Diabetic retinopathy (DR) is the most common microvascular complication of

diabetes and one of the main causes of blindness in adults, which greatly reduces the quality of life of diabetic patients. 45% of T2DM patients with a disease course of more than 10 years are associated with varying degrees of DR [10].

A large number of studies have shown that C-peptide can reduce the occurrence of DR. A retrospective analysis of the Diabetes Control and Complications Test showed that the residual secretion of serum C-peptide reduced the incidence of DR in T1DM patients. Studies have shown that serum C-peptide levels are negatively correlated with the prevalence of DR in patients with T2DM; DR patients show lower levels of fasting C-peptide, 2 h postprandial C-peptide, and ΔC-peptide (2h postprandial C-peptide minus fasting C-peptide) [11]. Retinal pigment epithelium (RPE) is a layer of epithelial cells containing pigment between the neural retina and the choroid; RPE cells have the potential to differentiate into photoreceptors and ganglion cells. The main cause of DR is damage to RPE, which leads to the destruction of the blood-retinal barrier and macular edema [12]. An animal experiment (STZ-induced T1DM mouse model) showed that C-peptide can improve retinal vascular permeability and prevent vascular leakage [13].

The mechanism by which C-peptide protects the retina is as follows: a) C-peptide improves retinal vascular permeability and prevents retinal neovascularization by affecting various effector proteins and transcription factors of RPE [13]. b) C-peptide normalizes Na^+-K^+-ATPase activity [14], on the one hand prevents RPE into fibroblasts and myofibroblasts [15], on the other hand inhibits the activity of retinal vascular endothelial growth factor dependent signaling pathway recovery [13], thereby preventing retinal detachment.

2.3. Effect of C-Peptide on Nervous System

Diabetic peripheral neuropathy (DPN) is the most common neurological complication of diabetic patients. DPN greatly increases the risk of diabetic patients suffering from foot ulcers, ankle fractures and lower limb amputations, leading to a significant decline in the quality of life of patients. It also causes a huge socio-economic burden.

C-peptide may have a potential inhibitory effect on the occurrence of DPN. A study of Chinese people showed that serum C-peptide was negatively correlated with the prevalence of DPN in T2DM patients; the serum concentration of fasting C-peptide, 2 h postprandial C-peptide and ΔC-peptide in the non-DPN group was significantly higher than that in the clinical DPN group and the confirmed DPN group [16]. Jolivalt *et al.* research showed that exogenous C-peptide can delay and prevent STZ-induced T1DM mice from experiencing movement, pain, temperature, and tactile dysfunction [17]. Clinical studies have shown that exogenous C-peptide can improve the ability of lower limb vibration perception in T1DM patients [18].

The mechanism by which C-peptide delays the occurrence of DPN is that C-peptide stimulates eNOS gene expression, enhances Na^+-K^+-ATPase activity,

and increases the secretion of neurotrophic factors, thereby protecting nerve cells and nerve fibers from high glucose toxicity damage [19].

2.4. Effect of C-Peptide on Macrovascular Disease

Diabetic macroangiopathy mainly includes coronary arteriosclerosis, cervical vascular and cerebral vascular sclerosis, renal arteriosclerosis, extremity arteriosclerosis, etc., which is the main cause of death in T2DM patients.

Studies have shown that serum C-peptide can promote arteriosclerosis. Pikkemaat *et al*. showed that increased baseline serum C-peptide concentration was associated with increased risk of all-cause death and cardiovascular death in newly diagnosed T2DM patients [20]. Further research shows that for all T2DM patients (regardless of whether they are newly diagnosed or not, regardless of whether they have atherosclerotic disease), C-peptide is positively correlated with cardiovascular mortality risk [21] [22].

The mechanism by which C-peptide promotes the occurrence of macrovascular complications is that C-peptide increases the lipid deposition on the vascular wall by increasing triglycerides or reducing high-density lipoprotein cholesterol levels; Increased risk of cardiovascular disease by promoting proliferation of vascular wall macrophages and vascular smooth muscle cells [23] [24].

3. The Role of C-Peptide in Other Aspects

3.1. Effect of C-Peptide on Reproductive Endocrine System

Non-alcoholic fatty liver disease (NAFLD) refers to a wide range and varying degrees of liver damage, from steatosis to steatohepatitis, advanced liver fibrosis and cirrhosis. C-peptide may promote the development of NAFLD. Data from the National Health and Nutrition Examination Survey show that fasting C-peptide is a risk factor for NAFLD in the general population of the United States [25]. Studies have shown that C-peptide is independently associated with liver fibrosis in T2DM patients [26]. The mechanism by which C-peptide promotes NAFLD is that C-peptide increases leptin by activating PI3K or PKB pathway [27], and the increase of leptin promotes hepatic steatosis [28].

C-peptide has a positive effect on the increase of bone mineral density (BMD). Studies have shown that C-peptide levels are positively correlated with lumbar BMD in postmenopausal women [29]. A study of postmenopausal women with diabetes has similar conclusions [30]. The mechanism by which C-peptide increases BMD may be through the ERK 1/2 pathway acting on osteoblasts, increasing collagen biosynthesis, and inhibiting osteoclasts by reducing RANKL expression [30].

C-peptide can maintain the reproductive system function of diabetic animals. Studies have shown that C-peptide can be combined with insulin (or even replace insulin) to prevent or delay mouse testicular dysfunction caused by diabetes [31]. The mechanism is as follows: a) The antioxidant effect of C-peptide reduces DNA breakage and apoptosis [32]; b) C-peptide can activate protein ki-

nase *α*, inhibit the generation of reactive oxygen species by mitochondrial NADPH oxidase, thereby preventing the apoptosis of endothelial cells [33].

3.2. C-Peptide Levels Are Associated with Hematological Diseases

C-peptide has a positive effect on the increase of hemoglobin. A cross-sectional study of T2DM patients showed that the concentrations of fasting C peptide, 2 h postprandial C-peptide, and *Δ*C-peptide in anemia patients were lower than those without anemia, indicating that the degree of anemia was negatively correlated with serum C-peptide concentrations in T2DM patients (fasting C peptide: $r = -0.057$, $p = 0.032$; 2 h postprandial C-peptide: $r = -0.098$, $p < 0.001$; ΔC-peptide: $r = -0.095$, $p < 0.001$) [34]. Hemoglobin is used as an indicator to evaluate the degree of anemia. The lower the hemoglobin, the more severe the anemia. At present, the mechanism of C-peptide on hemoglobin is not clear; it is considered that C-peptide may have the effect of anti-oxidation and maintenance of Na^+-k^+-ATPase activity, so as to prolong the life of red blood cells.

3.3. Effect of C-Peptide on Tissue Repair

C-peptide may have a potential role in promoting the repair of tissue damage. Studies have shown that C-peptide can significantly promote skin wound healing in diabetic mice [35]. A study of T1DM mice showed that when simvastatin induces skeletal muscle apoptosis, C-peptide can effectively inhibit apoptosis [36]. Tauber *et al*. study has shown that angiogenesis and granulation tissue formation in the early healing stage of non-diabeticrabbit skeletal muscle tissue samples are positively correlated with the level of C-peptide in peripheral blood [37], so it is believed that C-peptide can also be used to monitor the healing process of tissues without diabetes. The mechanism of C-peptide to promote tissue healing is as follows: a) C-peptide inhibits apoptosis through pertussis toxin sensitive pathway [36]. b) C-peptide induces endothelial cell migration and mediates neovascularization by activating extracellular signal-related kinases and inducing nitric oxide production [35].

3.4. The Role of C-Peptide in Neoplastic Diseases

C-peptide plays an important role in a variety of neoplastic diseases. C-peptide may have different effects on tumors in different parts. C-peptide can promote the occurrence and development of various tumors. A large prospective study showed that high levels of C-peptide increased the risk of developing cancer in the colon (OR = 1.73), liver (3.23), kidney, renal pelvis, and ureteral cancer (2.47) [38]. Another study showed that high levels of C-peptide may be associated with high-risk prostate cancer. The mechanism by which C-peptide promotes the occurrence of these tumors is not clear [39].

C-peptide may have an inhibitory effect on tumor formation and metastasis in certain parts. Studies have shown that C-peptide can prevent vascular leakage and metastasis of melanoma cells in the lungs of diabetic mice caused by hyper-

glycemia [40]. The mechanism may be that C-peptide reduces the damage of adhesion molecules to the vascular endothelium by inhibiting the induced intracellular events of vascular endothelial growth factor (including the increase of intracellular reactive oxygen levels, TGase2 activation, etc.), thereby preventing vascular leakage [40]. Research by Nogueira *et al*. Showed that C-peptide levels are inversely related to pancreatic duct adenocarcinoma in current smoking patients [41].

4. Outlook

In summary, C-peptide can play an active role in multiple systems, including chronic microvascular complications of diabetes, bone mineral density, reproductive system, anemia, tissue repair, and the occurrence of some tumors; however, it has adverse effects on diabetic macrovascular complications, fatty liver, and other tumors. At present, the many mechanisms of C-peptide are still unclear. At present, many mechanisms of action of C-peptide are still unclear; as a small molecule peptide, it is unclear whether it can cooperate with other substances to exert physiological and biochemical functions. With the deepening of research, C-peptide may be used as a breakthrough in diabetes treatment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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The Correlation between Hypertensive Intracerebral Hemorrhage and Internal Carotid Atherosclerosis Investigated by Carniocervial CTA

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Abstract

Objective: To investigate the relationship between hypertensive intracerebral hemorrhage and internal carotid atherosclerosis and its risk factors by CTA (Computed tomography angiography). **Methods:** The clinical materials of hypertensive intracerebral hemorrhage patients with carniocervial CTA from January 2018 to August 2019 in Puren Hospital of Wuhan were analyzed retrospectively. The correlation and risk factors between hypertensive intracerebral hemorrhage and internal carotid atherosclerosis were studied by logistic regression and descriptive analysis, at the same time, the application value of carniocervial CTA in patients with cerebral hemorrhage was evaluated. **Results:** There was a correlation between hypertensive intracerebral hemorrhage and internal carotid atherosclerosis ($\chi^2 = 5.319$, $P = 0.021 < 0.05$, OR = 2.70 > 1), which indicated that internal carotid atherosclerosis was the risk factor of hypertensive intracerebral hemorrhage, and there was no significant correlation between the location of internal carotid atherosclerosis, multiple atherosclerosis of internal carotid artery and hypertensive intracerebral hemorrhage. Monofactor analysis showed that the risk factors of hypertensive intracerebral hemorrhage with internal carotid atherosclerosis were sex, age, diabetes and hyperlipidemia. According to the logistic regression analysis, hyperlipidemia and diabetes were independent risk factors for hypertensive. **Conclusion:** The occurrence of hypertensive intracerebral hemorrhage is related to internal carotid atherosclerosis and is affected by many factors. Carniocervial CTA is helpful to the diagnosis of cerebral hemorrhage.

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Keywords

Hypertensive Intracerebral Hemorrhage, Internal Carotid Atherosclerosis, Computed Tomography Angiography

1. Background

Hypertensive intracerebral hemorrhage is a common cerebrovascular disease in clinical work. As the problem of aging population becomes more and more prominent, the incidence of the disease is also increasing year by year. It has the characteristics of disability rate, mortality rate, high recurrence rate and is one of the biggest threats to human health in the 21st century [1]. Studies have shown that carotid atherosclerosis exists in 20% of patients with intracerebral hemorrhage [2]. This paper uses head and neck CTA, to further explore the relationship between the characteristics of carotid atherosclerotic plaque in internal carotid artery and hypertensive intracerebral hemorrhage, and expounds the application value of CTA in intracerebral hemorrhage disease.

2. Objects and Methods

2.1. Object of Study

From January 2018 to August 2019, 50 patients with hypertensive intracerebral hemorrhage who were admitted to Puren Hospital of Wuhan City were selected as the experimental group and 50 patients with non-cranial hemorrhage were selected as the control group, including 9 patients with mild craniocerebral trauma, 20 patients with dizziness and headache, 21 patients with transient ischemic attack. All patients underwent CTA examination.

Inclusion criteria: 1) consistent with the clinical diagnostic guidelines related to hypertensive intracerebral hemorrhage [3]; 2) confirmed by MRI, CT and other imaging examinations; 3) initial onset.

Exclusion criteria: 1) patients with secondary intracerebral hemorrhage, such as aneurysm rupture, cerebrovascular malformation, trauma, tumor, etc.; 2) patients with recurrent hypertensive intracerebral hemorrhage; 3) patients with hemorrhagic cerebral infarction; 4) patients with head and neck CTA with poor imaging and incomplete clinical data.

From January 2018 to August 2019, 50 patients with non-cranial hemorrhage were selected as the control group, including 9 patients with mild craniocerebraltrauma, 20 patients with dizziness and headache, 21 patients with transient ischemic attack, and head and neck CTA were performed during hospitalization or during outpatient visits.

2.2. Methods

2.2.1. Instruments and Reagents

Adopt 128 row GE optima660CT machine, non-ionic contrast agent iodohydrol;

the patient has signed the consent before the examination.

2.2.2. Scanning Mode

Take the supine position, use the head frame to fix the head, ask the subject to keep the head and neck still during the examination, fix the forehead with bandages, ask the patient to breathe calmly, avoid swallowing when scanning. scanning range from the aortic arch to the cranial top. scan parameters: tube voltage 100 KV, tube current 350 mA, scan layer thickness, layer interval scan 0.6 mm scan field of view 400, LW = 40, pitch 0.984:1.000. Iodine sea-alcohol, iodine contrast agent g/50ml. 17.5 all patients were treated with low-dose group injection: through the elbow intravenous injection of contrast agent 15 ml and saline 20 ml flush tube, injection speed 4 - 5 ml/s, delay Xs (x represents the scan delay time, the routine set to 10 s) after the C4 vertebral lam dynamic scan. The micro-calcification region of interest (MROI) analysis software was used to observe and measure the peak concentration of the contrast agent at the C4 carotid artery, and obtain the turning point Y on the time-density curve of contrast enhancement Value, according to the conventional method (X + 2Y + 1) to calculate the enhanced scan delay time, wait for 5 min after injecting 50ml of contrast agent and 50 ml of saline to perform a two-phase scan of the same site with the contrast agent injection flow rate, and obtain a head and neck CTA image.

3. Basic Information

Record the patient's age, gender, diabetes, hyperlipidemia, smoking, drinking history and other data. Carotid atherosclerotic plaque is defined as local bulge thickening, protruding into the lumen, thickness ≥ 1.3 mm [4]. According to carniocervial CTA imaging data and the 7-segment method of internal carotid artery proposed by Bouthillieretal in 1996 [5], the carotid atherosclerosis was divided into extracranial atherosclerosis and intracranial atherosclerosis.

4. Statistical Processing

SPSS17.0 was used for statistical analysis. Counting data is expressed in (n%), using χ^2 test. The measurement data is expressed by (X ± S), and t test is used. Multivariate correlation analysis was performed using binary classification Logistic regression analysis. $P < 0.05$ is a significant difference, with statistical significance.

5. Result

5.1. Comparison of Basic Clinical Data

The patients in the hypertensive cerebral hemorrhage group were 36 - 81 years old, with an average of (62.86 ± 10.38) years old. There were 32 male patients and 18 female patients. The patients in the non-cerebral hemorrhage group were 27 - 82 years old, with an average of (59.86 ± 13.23) years old. There were 26 male patients and 24 female patients. Two independent sample t tests were car-

ried out on the ages of the two groups. The t value was 1.261 and the p value was 0.210. There was no difference in age between the two groups. Chi-square test was conducted on the gender composition of the two groups, and their χ^2 value was 1.478 and p value was 0.224. There was no difference in gender composition between the two groups. There was no statistically significant difference in age and gender between the two groups, and they were comparable.

5.2. Analysis of the Incidence of Internal Carotid Atherosclerosis in Patients with Hypertensive Cerebral Hemorrhage and the Correlation between Internal Carotid Atherosclerosis and Hypertensive Cerebral Hemorrhage

According to the imaging data of the patient's head and neck CTA, the statistics of internal carotid atherosclerotic plaque sclerosis. Among the 50 patients with hypertensive cerebral hemorrhage, 38 patients (76.0%) with internal carotid atherosclerosis, including 11 patients (28.9%) with internal carotid artery intracranial segment alone There were 6 patients (15.8%) with plaque in the extracranial segment of arteries, and 21 patients (55.3%) had both; in the control group, there were 27 patients (54%) with internal carotid atherosclerosis. Among them, 13 patients (48.1%) had plaque in the intracranial segment of the internal carotid artery alone, and 3 patients (11.1%) had plaque in the extracranial segment of the internal carotid artery (11.40%). The formation of internal carotid atherosclerosis is a risk factor for hypertensive cerebral hemorrhage ($P < 0.05$, OR == 2.70 > 1). There is no difference in the probability of plaques in the intracranial and extracranial segments of the internal carotid artery leading to cerebral hemorrhage ($P > 0.05$). Not relevant ($p > 0.05$). See Tables 1-3 for details.

5.3. Single Factor Analysis of Carotid Atherosclerosis in Patients with Hypertensive Cerebral Hemorrhage

Among 50 cases of hypertensive cerebral hemorrhage, patients with internal

Table 1. Correlation analysis of internal carotid atherosclerosis and hypertensive cerebral hemorrhage.

		With internal Carotid atherosclerosis	Without internal Carotid atherosclerosis	χ^2	P
Hypertensive cerebral hemorrhage	With	38	12	5.319	0.021
	Without	27	23		

Table 2. Correlation between different parts of internal carotid artery and hypertensive cerebral hemorrhage.

		Intracranial segment	Extracranial segment	χ^2	P
Hypertensive cerebral hemorrhage	With	32	27	0.340	0.560
	Without	24	14		

Table 3. Correlation between the features of internal carotid atherosclerosis site and hypertensive cerebral hemorrhage.

		Simple part	Mixed parts	χ^2	P
Hypertensive cerebral hemorrhage	With	17	21	1.332	0.248
	Without	16	11		

carotid atherosclerosis were divided into group A, and patients without internal carotid atherosclerosis were divided into group B. There are 38 people in group A and 12 people in group B. Univariate analysis showed that the risk factors for atherosclerosis in patients with hypertensive cerebral hemorrhage were gender, age, diabetes, smoking, hyperlipidemia and so on. The specific situation is shown in Table 4.

5.4. Multivariate Logistic Regression Analysis of Hypertensive Cerebral Hemorrhage

With or without hypertensive intracerebral hemorrhage (with hypertensive intracerebral hemorrhage = 1, without hypertensive intracerebral hemorrhage = 0) as the dependent variable, gender (male = 1, female = 0), age (≥60 is 1, <60 is 0), diabetes (yes = 1, no = 0), smoking (y = 1, no = 0), hyperlipidemia (y = 1, no = 0) and other factors are independent variables, and multivariate logistic is performed after assignment regression analysis. Hyperlipidemia and diabetes are independent risk factors for hypertensive cerebral hemorrhage. The specific situation is shown in Table 5.

6. Discussion

Atherosclerosis is a common cause of cerebrovascular disease. 70% to 80% of blood in the brain is supplied by the internal carotid artery. Previous studies have pointed out that carotid atherosclerotic plaque is a high factor for cerebral infarction. However, regarding carotid artery, there are few studies on the correlation between atherosclerosis and cerebral hemorrhage. This article uses the head and neck CTA to explain the correlation and risk factors of internal carotid atherosclerotic plaque with hypertensive cerebral hemorrhage.

This study found that the formation of internal carotid atherosclerotic plaques is a risk factor for hypertensive intracerebral hemorrhage, but it has no significant correlation with whether the plaques are in the intracranial or extracranial segment of the internal carotid artery. Hypertensive cerebral hemorrhage is based on hypertension, and various types of hypertension can cause cerebral hemorrhage under certain circumstances. At the same time, studies have shown that the incidence of atherosclerosis in patients with hypertension is significantly increased. Patients with hypertension have atherosclerosis 3 - 4 times higher than those with normal blood pressure. May be due to high blood pressure, the arterial wall is under high pressure, increasing the excitability of its sympathetic

Table 4. Single factor analysis of carotid atherosclerosis in patients with hypertensive cerebral hemorrhage.

relevant factor	classification	Group A	Group B	χ^2	P
Gender	Male	28	4	4.813	0.028
	Female	10	8		
Age	≥60	28	3	7.225	0.007
	<60	10	9		
Diabetes	With	22	2	6.211	0.013
	Without	16	10		
Hyperlipidemia	With	30	5	4.31	0.036
	Without	15	7		
Smoking	With	27	3	6.255	0.012
	Without	11	9		
Drinking	With	19	8	1.020	0.313
	Without	19	4		

Note: Group A: patients with internal carotid atherosclerosis among 50 cases of hypertensive cerebral hemorrhage; Group B: patients without internal carotid atherosclerosis among 50 cases of hypertensive cerebral hemorrhage.

Table 5. Multivariate logistic regression analysis of hypertensive cerebral hemorrhage.

	B	S.E,	Wals	df	Sig.	Exp (B)	EXP(B) 95% C.I.
Gender	0.053	0.469	0.013	1	0.909	1.055	0.042 - 2.647
Age	−0.284	0.480	0.352	1	0.553	0.752	0.294 - 1.926
Hyperlipidemia	0.916	0.449	4.163	1	0.041	2.500	1.037 - 6.027
Diabetes	1.221	0.499	5.996	1	0.014	3.391	1.276 - 9.010
Smoking	0.526	0.445	1.397	1	0.237	1.692	0.707 - 4.050
constant	−1.075	0.489	4.841	1	0.028	0.341	

nerves, increasing the amount of adrenaline angiotensin, endothelial cell damage, causing excessive prostaglandins and thromboxane to adhere to the patient's platelets And lipid deposition, and stimulate smooth muscle cell proliferation, eventually leading to the formation of carotid atherosclerosis [6]. In this study, it was found that in the hypertensive cerebral hemorrhage group and the control group, the incidence of atherosclerosis in the intracranial segment of the internal carotid artery was higher than that in the extracranial segment, respectively, 64% and 48%. The specific reason is not clear, and it may be caused by the relatively tortuous blood vessels of the internal carotid artery from the rock section to the communication section in the cranial, resulting in relatively unstable hemodynamics.

Multivariate analysis of whether patients with hypertensive cerebral hemorrhage have internal carotid atherosclerosis found that age greater than 60 years

old, hyperlipidemia, diabetes, smoking, and gender are all influencing factors. And multivariate Logistic regression analysis of hypertensive cerebral hemorrhage pointed out that hyperlipidemia (OR = 2.500, 95% CI: 1.037 - 6.027, P = 0.041) and diabetes (OR = 3.391, 95% CI: 1.276 - 9.010, P = 0.014) is an independent risk factor for hypertensive cerebral hemorrhage. Therefore, for hypertensive patients over 60 years old, in addition to actively controlling blood pressure, blood lipid and blood sugar levels should also be paid attention to. It has positive significance for preventing hypertensive cerebral hemorrhage.

With the development of CT angiography (Computed tomography angiogram, CTA) technology, we can use CTA image data to observe and measure the plaque in the blood vessels of the head and neck and related data. Some research results show that in detecting the number of internal carotid artery plaques, the results of ultrasound examination are significantly lower than the results of CTA examinations. The relationship between the characteristics of plaque and hypertensive cerebral hemorrhage. CT angiography of the head and neck is one of the important examination methods of cerebrovascular disease. Its operation is simple, the result is faster, safe and efficient, and it can accurately display the position, size, and shape of the examiner's blood vessel and surrounding tissue. The doctor provides a reliable basis for the diagnosis of the disease [7]. For patients with cerebral hemorrhage, CTA examination can further investigate cerebral hemorrhage caused by aneurysm and arteriovenous malformation. Compared with ultrasound, the imaging of blood vessels by CTA is clearer, especially for the examination of intracranial blood vessels.

7. Conclusion

In summary, patients with high blood pressure and diabetes who are over 60 years old are actively controlling blood lipid levels and quitting smoking, which is one of the important measures to prevent hypertensive cerebral hemorrhage. Reducing the prevalence of carotid atherosclerosis has certain significance for controlling hypertensive cerebral hemorrhage. As an auxiliary examination method of cerebrovascular disease, CTA is conducive to improving the accuracy of diagnosis and is worthy of promotion in the clinic.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Study on the Effects of Different Dietary Preparation Methods on Capsule Endoscopy

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Abstract

Background: Capsule endoscopy (CE) examination has become the first-line diagnostic method for small bowel disease, and intestinal preparation is a key factor affecting the quality of CE examination. At present, there is no uniform standard for dietary preparation methods before CE at home and abroad, and there are few systematic comparative studies on the choice of dietary methods. **Objective:** To explore the best method of preparing diet for capsule endoscopy (CE) examination. **Methods**: 93 Patients who underwent CE examination in the digestive endoscopy center of Wuhan union medical college hospital in October 2019 were randomly divided into 3 groups. Group A (n = 31): 1 day before the examination; a diet with low-residue was given, and 1 day before the examination, fasting was started at 8 p.m. until the examination was completed. Group B (n = 30): a clear liquid diet was followed 1 day before the examination, and fasting was started at 8 p.m. 1 day before the examination until the examination was completed. Group C (n = 32): followed an ordinary diet 1 day before the examination, and began fasting at 8 p.m. 1 day before the examination until the examination was completed. Intestinal preparation of the same drug was performed in 3 groups, and the incidence of side effects and intestinal cleanliness of each group were compared. **Results:** There was no significant difference in cleanliness between the three groups ($P > 0.05$). The incidence of side effects in the three groups was 28.13%, 70.00% and 21.88%, respectively. The difference between group A and group B, group C and group B is statistically significant ($P < 0.05$). **Conclusion:** The three diet preparation methods of low-residue diet, clear liquid diet and ordinary diet can all achieve good intestinal cleansing effect. Clear liquid diet can not obviously improve the cleanliness of the small intestine, and there are relatively many side effects.

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Keywords

Capsule Endoscopy, Intestinal Preparation, Diet

1. Introduction

Capsule endoscopy (CE) examination has the advantages of no pain, simple operation, safety, non-invasiveness, and no cross infection, which greatly expands the field of vision of digestive tract examination. With the rapid development of gastrointestinal endoscopy technology, small intestine capsule endoscopy has become the preferred method for diagnosing small intestinal diseases in the field of digestive diseases. However, there are still some shortcomings in the endoscopy of small intestine capsules. Intestinal contents play a decisive role in the accuracy of diagnosis.

Intestinal preparation is a key element that affects the quality of CE examination [1] [2] [3]. If the gastrointestinal tract is not cleaned thoroughly, and the contents of the intestinal tract remain, it may result in unsatisfactory captured images, which may affect the examination results and even cause misdiagnosis and missed diagnosis. Therefore, thorough gastrointestinal cleansing before capsule endoscopy is performed to improve the clarity of the captured images, which is helpful to improve the accuracy of small bowel disease detection.

Dietary preparation is an important auxiliary measure for intestinal preparation [4] [5]. According to the amount of residue generated, dietary preparation can be divided into clear diet, less residue diet and ordinary diet. At present, there is no uniform standard for dietary preparation methods before CE at home and abroad, and there are few systematic comparative studies on the choice of dietary methods. In order to find out the best diet preparation methods before CE examination, this article compares and studies the differences in the effect of different diet preparation protocols on capsule endoscopic bowel preparation.

2. Materials and Methods

2.1. General Information

The case data were included in 93 patients who underwent capsule endoscopy in the digestive endoscopy center of wuhan union medical college hospital in October 2019, including 60 males and 33 females aged 15 - 89 years. Exclusion criteria: (1) failure of total intestinal examination caused by device factor; (2) the capsule lens did not reach the small intestine and the capsule lens did not complete the examination; (3) gastrointestinal bleeding, eating in advance and other factors interfere with the examination of small intestine cleanliness.

2.2. Methods of Examination

(1) Inform the subjects and their families of the precautions for CE examination,

and sign the informed consent. (2) Preparation of intestinal tract: Before the examination, group A (n = 31), group B (n = 30) and group C (n = 32) were given a slag-free diet, a clear-liquid diet and a normal diet, and the rest were the same. That is to say, fasting will be started at 8:00 p.m. before the inspection until the inspection is completed; at 9:00 p.m. before the inspection on the 1st before inspection, one packet of compound polyethylene glycol (PEG, Fujingqing) will be dissolved in the morning at 5:00 and 6:00 on the inspection day. Put it in 1 L of warm water and finish it in 1 hour. On the day of the inspection at 7:00, a bottle of simethicone is dissolved in 200ml of cold water and finished in 5 minutes. (3) Inspection: After swallowing CE with a small amount of water, the subjects are instructed to fast and not to stay away from the inspection site. The doctor monitors the operation of the capsules in real time and informs them to eat according to the monitoring results.

2.3. Evaluation Method

Observe the cleanliness of the small intestine, calculatethe incidence of side effects. Small intestine cleanliness is evaluated by a single-blind method, which is scored by CE readers based on the cleanliness of the intestinal tract of the CE image and the overall observation effect. Intestinal cleaning grading: Level 1: there are very few bubbles in the small intestine, no residue and turbid liquid. The observation effect is good; Level 2: there is a small amount of air bubbles or turbid liquid in the small intestine, no residue, which has little impact on the observation effect; Level 3: a large amount of air bubbles or turbid liquid is found in the small intestine, and a little residue, which affects the observation effect; Level 4: a large amount bubbles and turbid liquid, more residue gathered in the small intestine, which seriously affect the observation. Side effects include gastrointestinal symptoms such as abdominal pain, nausea, and vomiting, as well as general malaise such as dizziness and palpitation. The incidence of side reactions is the percentage of people with side reactions in the total number of people.

2.4. Statistical Methods

SPSS 22.0 statistical software was used for statistical analysis. The small intestine cleanliness was measured by the rank sum test, the measurement data were expressed by mean ± standard deviation ($x \pm s$), and the incidence of side reactions was measured by chi-square test of line and column list data, with the test standard = 0.05.

3. Results

3.1. Comparison of General Conditions of Patients

General statistics of gender and age of patients in the three groups: there was no significant difference in gender and age among the three groups, and the groups were comparable (Table 1).

Table 1. Comparison of general conditions of patients.

Groups	n	Gender		Age (years)
		M	F	
Group A	31	18	13	42.77 ± 15.34
Group B	30	21	9	43.77 ± 12.47
Group C	32	21	11	43.00 ± 13.24

3.2. Comparison of the Three Groups Related Indicators

There was no significant difference in cleanliness of small intestine between groups A, B and C ($P > 0.05$). The results showed that during CE examination, taking a sufficient amount of laxatives, most can achieve good intestinal preparation, but the relationship with dieting seems not so close. Among the 37 patients, 11 had abdominal pain and distension, 7 had nausea and vomiting, and 19 had palpitation, dizziness and chills. The incidences of side effects in groups A, B, and C were 28.13%, 70.00%, and 21.88%, respectively, with statistically significant differences between group A and B, C and B ($P < 0.05$). The results showed that there were significant differences in side effects such as abdominal pain, nausea, or dizziness in the normal diet group and the low-residue diet group compared with the clear liquid diet group. See **Table 2**.

4. Conclusion

The three diet preparation methods of low-residue diet, clear liquid diet and ordinary diet can all achieve good intestinal cleansing effect. Clear liquid diet can not obviously improve the cleanliness of the small intestine, and there are relatively more side effects. The key part of intestinal preparation before capsule endoscopy is cleaning the intestines. Taking a sufficient amount of laxatives strictly according to doctor's instructions until clear liquid is excreted, most can achieve good intestinal preparation effects. And the relationship between cleanliness and dieting seems not so close. Furthermore, patients have fewer side effects after eating, and will be more tolerant to capsule endoscopy examination.

5. Discussion

Since the capsule endoscopy was introduced in 1999, the clinical application of capsule endoscopy has developed rapidly [6], and capsule endoscopy has now become the preferred diagnostic method for small bowel diseases [7]. However, due to the lack of water injection, gas injection, suction, and controllable functions of the capsule endoscope, the real-time intervention of the shooting field cannot be performed, so the CE examination has higher requirements for cleanliness of the small intestine [8] [9]. In addition to the quality of intestinal preparation and oral laxatives, it is closely related to diet preparation programs [10] [11]. There are few systematic comparisons of diet preparation methods before the CE examination. At present, it is generally believed that the effect of a clear liquid diet is better, but it is unclear whether the clear liquid diet before the test

Table 2. Comparison of the three groups related indicators.

Groups	Cleanliness of small intestine/case				The incidence of side reactions/case (%)
	Level 1	Level 2	Level 3	Level 4	
Group A (n = 31)	5	14	8	2	9 (29.03)
Group B (n = 30)	7	14	6	3	21 (70.00)
Group C (n = 32)	4	15	10	3	7 (21.88)

has sufficient benefits to patients compared with low-residue diet and the normaldiet [12] [13] [14] [15].

Intestinal preparation is one of the key aspects of capsule endoscopy. At present, it mainly focuses on cleaning the intestines and preparing food. We use 3 L PEG electrolyte intestinal lavage liquid for intestinal preparation, and the effect is satisfactory [16]. As for diet preparation, some are accustomed to restricting their diet 1 d before the examination and fasting from 8:00 p.m. the day before the examination. Due to lack of energy and nutritional supplements, the tolerance of the testers is significantly decreased, especially in poor health or with basic diseases. The elderly, or outpatients from afar, are more likely to have hypoglycemia-like reactions, aggravate discomfort, and directly affect the examination results. We observed the intestinal cleanliness of the three groups with different dietary conditions, and the results were statistically analyzed, with $P > 0.05$. There was no statistical difference in intestinal cleanliness among the three groups. The three diets had little effect on capsule endoscopy. A clear liquid diet cannot significantly improve the cleanliness of the small intestine, and there are relatively more side effects. There are still some deficiencies in this study, we need to expand the sample size and do more detailed researches. And it's necessary to do systematic comparative studies on the choice of dietary methods in the following time.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Construction of the First Certification Evaluation Index System for Diabetes Specialist Nurses by Delphi Method

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Abstract

Objective: To establish an evaluation index system for the first-time certification of diabetes specialist nurses in line with the clinical nursing practice in China, and to provide a reference basis for government administrations and hospitals to formulate unified standards. **Methods:** After the research team has determined the theme, it will consult the development of A) specialist nursing through the library, Internet and electronic literature database; B) the history of the development of specialist nurses in diabetes and other fields; C) the selection criteria of specialist nurses; D) the training of specialist nurses system, assessment method; E) Specialist nurse certification body, evaluation standards and certification standards and other related documents, literature, books and report materials. The author established the preliminary index system through literature review, specialist interview and group discussion and used Delphi method to organize three rounds of 30 experts' consultations. **Results:** A first certification evaluation index system including 8 fundamental indicators, 5 first-level indicators, 19 second-level indicators and 99 third-level indicators was constructed for diabetes nurses. **Conclusion:** The results of three rounds of expert consultation and demonstration are reliable, and the constructed index system is suitable for comprehensive evaluation of diabetes specialist nurses, which provides a basis for the effective management of nursing resources.

Keywords

Diabetic Specialist Nurses, First Certification Evaluation Index, Delphi Method

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1. Introduction

With the development of social economy, the prevalence of diabetes is rising world widely. According to the statistics of the International Diabetes Federation (IDF), it is estimated that 380 million people will be diagnosed with diabetes by 2025 [1]. A survey conducted by the Diabetes Branch of the Chinese Medical Association, published in the New England Journal in March 2010, shows that the number of patients with diabetes in China has exceeded 92 million [2], making it a major diabetic country. In order to cope with the growing population of diabetic patients and the resulting economic and medical burdens, diabetes specialist nurses have been trained throughout the country [3]. However, there is no uniform standard for the selection, training, assessment and certification of diabetes nurses in China so far [4] [5]. In this study, the Delphi method was used to establish an evaluation index for the first certification of diabetes specialist nurses, which is expected to provide a reference basis for the certification of diabetes specialist nurses.

2. Steps and Methods

2.1. Set up a Research Team and Select Consulting Experts

The research team consisted of 5 people, including 1 director of nursing department, 1 head nurse, and 3 graduate students. 30 clinical experts, involving nursing management, nursing education, clinical nursing, community nursing, medicine, were selected from 10 provinces in East China, South China, West China, North China, and Central China. Every expert is either from Class A First Class Hospital or from college of advanced nursing with the technical title of deputy senior or above (except the experts from clinical nursing, whose minimal title is intermediate level). The experts with bachelor degree or above have more than 10 years of experience in diabetes or related majors.

2.2. Research Tools

After the research team has determined the theme, it will consult the development of A) specialist nursing through the library, Internet and electronic literature database; B) the history of the development of specialist nurses in diabetes and other fields; C) the selection criteria of specialist nurses; D) the training of specialist nurses System, assessment method; E) specialist nurse certification body, evaluation standards and certification standards and other related documents, literature, books and report materials. The author established the preliminary index system through literature review, specialist interview and group discussion and used Delphi method to organize three rounds of 30 experts' consultations. Key words of database retrieval include: diabetes specialist nurses, current situation and development of specialist nursing, selection criteria, system specifications, assessment methods, certification bodies, evaluation criteria, etc. Review the collected literature content (see **Figure 1** for the selection process of literature data and **Table 1** for the selection content).

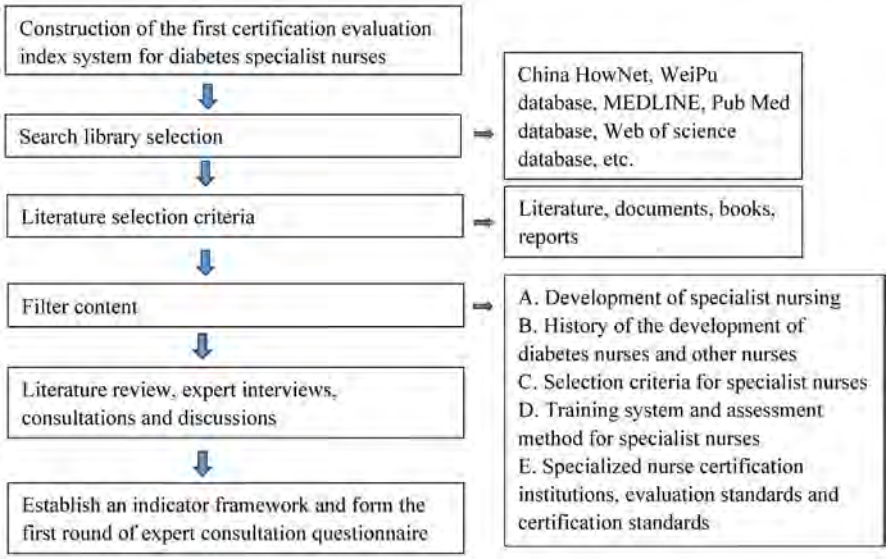

Figure 1. Selection process of research materials.

Table 1. Research materials of literature review and selection.

No.	Literature review
1	Junwen Zhao, Yajie Li. Effective management of clinical nursing experts and specialized nurses [J]. Nanfang Journal of Nursing, 2005A4: A33, (03): 21-23.
2	Liping Wu, Huiping Li, Mei Zhao Xiuping Fang. Status quo of diabetes specialist nurses cultivation. Journal of Nursing Science. 2011. (11): 87-90
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The first round of expert consultation questionnaire was formed based on literature review and expert interviews. The questionnaire contains three parts: the basic information of the expert, the text of the questionnaire comprising of the importance of indicators and the recommended modification by the expert as well as the familiarity of the expert with the question and the main basis for the expert to judge. The importance of indicators is based on the Likert scoring method, which is from very important to unimportant (1 - 5 points, respectively) [6]. Experts' familiarity with various indicators is divided into 5 levels: unfamiliar, not very familiar, general, relatively familiar, very familiar. Three rounds of questionnaires were distributed and received by email.

2.3. Statistical Methods

The results were processed and analyzed by Microsoft Excel 2003 and SPSS17.0 to establish a database. The questionnaire response rate (%) is used to reflect the enthusiasm of experts. The degree of coordination of expert opinions is expressed by the coefficient of variation (Vj) of the evaluation results of various indicators and the Kendall's W coefficient of expert opinions and its significance test, where Vj represents the degree of fluctuation of the relative importance of the indicators, and the smaller the Vj, the higher degree of coordination of expert opinions; The coordination coefficient (W) ranging between 0 - 1 is used to test whether the opinions of experts on the indicators are consistent, the larger the value, the higher the coordination of the opinions of the experts. Significance test was performed on W, if $P < 0.05$, it means that the experts have consistent scores on the indicators, indicating the results are desirable. The selection of research indicators is based on the criteria of satisfying both the mean value of importance value > 3.50 and the coefficient of variation ≤ 0.25. At the same time, combined with expert opinions, the indicators are screened by the collective review of the research team.

3. Results

3.1. Basic Situation of Consulting Experts

A total of 30 consulting experts participated, 17 of them aged 40 - 49 years old, accounting for 56.7%. 90% of the experts had more than 20-year working experience and had professional titles as deputy senior or higher. All of them had bachelor's degree or above including 11 professors (36.7%). There were 15 experts major in clinical nursing (50%) and 8 experts major in nursing management (26.7%). Except for one from nursing colleges, the remaining 29 were from the Class A First Class Hospitals.

3.2. The Questionnaire Recall Situation

30 questionnaires were distributed each round, and the first round had the effective response rate 100%; the second round, 86.7%; the third round, 100%.

3.3. The Degree of Authority of Experts

The degree of expert authority is reflected by the basis of experts' judgment on indicators and the familiarity of experts with indicators [7] [8] [9]. The average authoritative degree of experts in three rounds of consultation were 0.77 - 0.94, 0.87, 0.87, respectively. It is generally considered that the degree of expert authority ≥ 0.7 is acceptable [7]. Thus, the degree of expert authority in this study is relatively high.

3.4. The Degree of Concentration of Expert Opinions

The degree of concentration of expert opinions is expressed by the mean and standard deviation of importance assignment. The larger the mean, the more

important the indicator is. The average values of the indicators in the three rounds of investigation were: 3.38 - 4.93, 2.67 - 5.00, 2.90 - 5.00; and corresponding standard deviations were: 0.13 - 0.91, 0.10 - 1.06, 0.00 - 1.01, respectively.

3.5. Degree of Coordination of Expert Opinions

After three rounds of consultation, the coefficient of variation of the third round of index assignment was 0.00 - 0.38. The Kendall harmony coefficients W of experts in the three rounds of consultation were 0.366, 0.374, and 0.382, respectively, and the significance test $P < 0.01$ indicated that the experts' opinions were well coordinated, and the results were desirable.

3.6. Expert Consultation Results

According to the selection criteria of the indicators and discussion by the research group, the indicators that failed both directions were deleted, and the indicators that failed one way were included in the next round of continued discussion. Due to space limitations, only the first two rounds of results were attached (Table 2).

4. Discussion

4.1. Representativeness of Experts and Reliability of Prediction

The selection of experts plays a key role in the Delphi method. In the study, we fully considered the professional representativeness, academic authority and geographical representation of experts. The selected experts include East China, West China, South China, North China, and Central China; their research areas include clinical nursing, nursing management, nursing education, community nursing and clinical medicine. The experts have a high level of knowledge and years of working experience in their professional fields. Many experts serve as Master or PhD supervisor. Moreover, the average authoritative coefficient, coordination coefficient and consistency of experts are relatively high; therefore, the representativeness and predictability of experts in this study are satisfied.

4.2. Analysis of the Evaluation Index System for the First Certification of Diabetes Specialist Nurses

Diabetes specialist nurses are nursing experts, and thus it requires that they should have certain professional abilities. Educational background and professional title are important factors that reflect the theoretical knowledge level and clinical professional skills of clinical nurses [8]. The years of working experience as a diabetes specialist can indirectly reflect the accumulated experience of the diabetes specialist nurses. The computer skill is a necessary part of the knowledge structure of expert talents. Nursing scientific research is an important means to promote the development of nursing science and to improve the quality of clinical nursing [9] [10] [11]. Specialized nurses conduct evidence-based

Table 2. Mean value and coefficient of variation of the first and second round evaluation indicators.

Indicators	First round		Second round	
	Importance score	Coefficient of variation	Importance score	Coefficient of variation
	$\bar{x} \pm s$	Vj	$\bar{x} \pm s$	Vj
J1 Nurse practice certificate	4.93 ± 0.26	0.05	5.00 ± 0.00	0.00
J2 Age	*3.38 ± 0.98	*0.29	-	-
J3 Education and degree	3.97 ± 0.73	0.18	3.76 ± 0.62	0.17
J4 Job title	3.76 ± 0.79	0.21	3.67 ± 0.80	0.22
J5 Diabetes specialist working years	4.72 ± 0.53	0.11	4.43 ± 0.75	0.17
J6 Further study experience	4.07 ± 0.96	0.24	4.00 ± 0.89	0.22
J7 Annual paper published in recent 5 years (above statistic sources)	3.66 ± 0.97	*0.27	3.58 ± 0.98	*0.26
J8 Scientific achievement	3.62 ± 0.94	*0.26	*2.90 ± 1.09	*0.38
J9 Funded research	3.65 ± 0.95	*0.26	3.54 ± 0.85	*0.27
J10 English proficiency	3.62 ± 1.05	*0.27	3.59 ± 0.90	*0.26
J11 Computer skill	3.79 ± 0.90	0.24	3.62 ± 0.92	0.25
J12 Continuing education credits	3.69 ± 1.17	*0.32	*3.41 ± 0.26	*0.30
J13 Expert recommendation	3.59 ± 1.09	*0.30	3.71 ± 0.26	*0.26
I Professional attitude	4.93 ± 0.26	0.05	4.95 ± 0.22	0.03
II Service awareness	4.93 ± 0.26	0.05	4.86 ± 0.36	0.07
II Professionalism	4.83 ± 0.38	0.08	4.90 ± 0.30	0.06
II Team spirit	4.76 ± 0.44	0.09	4.90 ± 0.30	0.06
II Enterprising spirit	4.55 ± 0.51	0.11	4.71 ± 0.46	0.10
I Professional knowledge	4.90 ± 0.31	0.06	4.88 ± 0.41	0.03
II Basic knowledge	4.72 ± 0.45	0.10	4.95 ± 0.22	0.04
II Specialist knowledge	4.90 ± 0.31	0.06	4.95 ± 0.22	0.04
II Humanities and social sciences and other related knowledge	4.10 ± 0.86	0.21	4.48 ± 0.75	0.17
I Specialist technology	4.86 ± 0.44	0.09	4.76 ± 0.51	0.04
II Nursing assessment technology	4.76 ± 0.44	0.09	4.76 ± 0.44	0.09
II Nursing implementation technology	4.79 ± 0.41	0.09	4.86 ± 0.36	0.07
II Specialist technical cooperation	4.55 ± 0.69	0.14	4.71 ± 0.56	0.12
II Instrumentation technology	4.55 ± 0.63	0.15	4.52 ± 0.60	0.13
I Core capabilities	4.69 ± 0.54	0.12	4.72 ± 0.45	0.09
II Direct patient caring	4.69 ± 0.54	0.12	4.86 ± 0.36	0.07
II Scientific research	4.00 ± 0.89	0.22	4.86 ± 0.48	0.10
II Nursing management	3.90 ± 0.86	0.22	4.10 ± 0.07	0.17
II Nursing education	4.69 ± 0.54	0.54	3.86 ± 0.79	0.21
II Consultation	4.52 ± 0.63	0.14	4.57 ± 0.68	0.15
II Ethical decision-making	4.10 ± 0.90	0.22	4.24 ± 1.04	0.25
I Personal qualities	4.31 ± 0.76	0.18	4.43 ± 0.54	0.12
II Physical fitness	4.34 ± 0.67	0.15	4.57 ± 0.51	0.11
II Psychological health	4.41 ± 0.68	0.15	4.62 ± 0.50	0.11

Note: * indicates that it does *not* meet the criteria for index screening.

nursing research on new evidence found to continuously improve nursing practice [12]. However, according to the actual situation in China, the overall English level and scientific research level are limited. Even graduated from the nursing department, they have difficulties in communication in English resulting in that they can only obtain the latest foreign knowledge and information indirectly [13]. Thus, treating "Funded research" and "English proficiency" as fundamental indicators is not desirable, but in view of their importance, we decided to include the "annual average paper publication status", which is relatively easier, and the "English proficiency" is listed as one of the first-level indicators.

The construction of this research indicator framework is guided by the "Attitude Skill Knowledge" (ASK) theory. The ASK theory [14] [15] was proposed by professor Mayo from Harvard University in the 1860s and he pointed out that there is a progressive relationship among knowledge, attitudes and skills, in which knowledge is the foundation of skills; attitude is the motivation to acquire skills, and acquisition of skills is the goal. The first-level indicators of this study not only cover professional attitudes, professional knowledge, professional skills, and core competencies, but also consider personal characteristics; in the second-level indicators, there are specific requirements for each item, which can be used to systematically and comprehensively evaluate diabetes specialist nurses. In terms of professional attitude, nursing is the most humanized profession. Only people with love and service consciousness can be qualified for this noble profession. In terms of professional knowledge, diabetes specialist nurses are nursing experts in the field of diabetes, and they are required not only to master precise and specialized nursing theory, basic knowledge, and specialist knowledge, but also to be familiar with the frontier knowledge in the field of diabetes, health policies, organizational management, health economics and social sciences. IN terms of professional technology, a diabetes specialist nurse should not only master the nursing skills of this specialty, but also master the use of diabetes related instruments and the skills of cooperation with other medical personnel to provide systematic, comprehensive and holistic care for patients.

In terms of core competence, in order to enable diabetes specialist nurses to be competent for different tasks, including disease prevention, health promotion, and nursing of acute and chronic diseases, it is necessary to cultivate the professional attitude, professional knowledge and technology of diabetes specialist nurses. As for personal characteristics, nursing tasks are heavy, and only a nurse with a healthy body can be competent. When dealing with pressure from work and life, they should reasonably control their emotions, adjust themselves, fully understand themselves and make appropriate evaluations of themselves.

4.3. Suggestions on the Implementation of the First Certification Evaluation Index System for Diabetes Specialist Nurses

1) It is necessary to establish relevant systems and legal regulations for the certification of diabetes specialist nurses. At present, the current status of the practice of diabetes specialist nurses in our country is neither compliant nor

rule-based [16]. Only by guaranteeing the legal certification of diabetic specialist nurses and having the law to be able to guarantee the healthy operation of the diabetic nurse certification mechanism, should the government be given full play to standardize the management of diabetic nurses and ensure the quality and control of diabetic nurses.

2) It is also necessary to encourage the establishment of statutory social intermediary institutions, which are entrusted or authorized by the government to certify diabetes specialist nurses and other specialist nurses. It is an independent non-profit organization between the government, society and medical units. The organization can be scientific groups or professional associations. The legal social intermediary organization can guarantee the objectivity and fairness of the certification and evaluation of diabetes nurses.

3) It is recommended to formulate the first certification procedure for diabetic specialist nurses according to the following procedures: a) An individual submits a certification application to the relevant certification organization with corresponding application materials; b) The certification organization conducts the qualification review of the applicant according to the certification standards; c) Applicants are required to conduct theoretical assessment; d) Interview; e) Comprehensive evaluation of clinical ability; f) Issue of diabetes specialist nurse certificate. Standardized first-time certification procedure of diabetes specialty nurses can make the certification of nurses more convenient and standardized.

The government should strengthen the selection of certified experts, closely connect academic experts. In additional to management authorities and industry, other parties should participate and cooperate with one another in order to give full play to their respective advantages. To strengthen the combination of certification and the practice registration system, we must not only allow practice registration to promote the development of professional certification, but also reflect that certification is the basis for the effective implementation of the practice registration system.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Positional Changes of the Hyoid Bone after Correction of the Glosso-Larynx—Hyoid Bone Complex (HBC)

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Abstract

Human hyoid bone floats in the neck. Downwards of an amount of about half vertebra and 0.3 cm forward following the correction of the glosso-larynx (CGL) was observed by simple XP. Disappearance of symptoms of the ankyloglossia with deviation of the epiglottis and larynx (ADEL) and increased of body temperature were observed after CGL. The results suggest that hyoid bone makes a complex that controls not only respiration and swallowing but also controls metabolism. It might be called hyoid bone complex (HBC).

Keywords

Respiration, Swallowing, Metabolism, Homo Neanderthalensis

1. Introduction

Symptoms and signs of ankyloglossia with deviation of the epiglottis and larynx (ADEL) are caused by respiratory insufficiencies. Correction of the glosso-larynx (CGL) is a surgery for ADEL, consisting in cutting the frontal bundles of genioglossus muscles. Following CGL, the deviated epiglottis and larynx move downwards aligned with the choanae, and improved respiratory rate and pulmonary function and increased body temperature are observed. The possible mechanisms leading to improved respiratory rate and increased body temperature cannot be explained by the cut of the bundles of genioglossus muscle alone and additional factors are likely to play a role, for example positional changes of the hyoid bone occurring after CGL [1] [2] [3] [4] [5].

The aim of this study was to assess the positional changes of the hyoid bone following GCL by using simple X-ray photographs (XPs) in order to assess the possible mechanisms at the level of the hyoid bone that may be related to the

beneficial effects observed following CGL.

2. Method

A total of 232 patients (138 females, 94 males; 17 to 78 years old (yr), average 40.1 yr) that underwent CGL from 2013 to 2016 were studied by taking XPs before and after CGL (Figure 1).

To assess possible positional changes in the hyoid bone, two measures were extracted from XPs and compared before and after CGL. Specifically, we measured the height of point V and the length of segment VH, defined in the followings. A horizontal line was drawn to connect the outermost part of the hyoid bone (point H) to the vertebrae (point V) and the distance between the two points was computed (length of segment VH), as shown in Figure 1.

To define the height of point V along the vertebrae, four possible positions were considered on each vertebra: the upper part (e.g., IIIup), the central part (e.g., III) the lower part (e.g., IIIdn) and the joint between two vertebrae (e.g., III/IV).

The following two measures were assessed and compared before and after CGL:

1) The height of point V.

2) The length of segment VH.

For the sake of comparing the length of segment VH before and after CGL, calibrations on the XPs were performed to each film according to the scale on film.

Possible differences in mean height of point V in the study sample before and after CGL were assessed by using a contingency table test (StatView 4.5) and possible differences in mean length of segment VH before and after CGL were addressed by using Student t-tests (Microsoft Exel Mc2011).

3. Results

3.1. Distribution of Height of Point V

The height of point V before CGL ranged from the third vertebra (III) to the

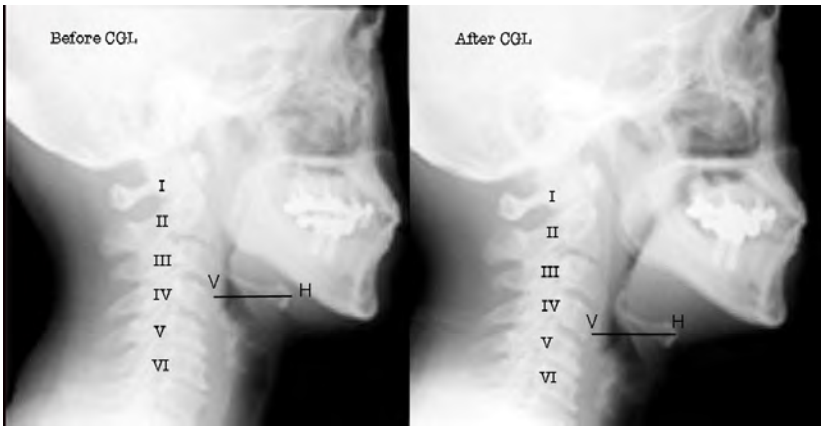

Figure 1. XPs in one of the participants before and after CGL.

upper part of the fifth vertebra (Vup). After CGL the heights of point V shifted, on average, towards lower vertebrae and ranged from the third (III) to the fifth vertebra (V). The median value of the height of point V before and after CGL was IV up and IV, respectively, suggesting a trend towards a downward shift (Figure 2).

Statistical analysis showed that there was a significant difference between two distributions ($p < 0.0001$), suggesting a tendency to a downward shift in the height of point V following CGL.

3.2. Length of Segment VH

The average length of segment VH before and after CGL was 4.4 cm (s.d. 0.63) and 4.7 cm (s.d. 0.64), respectively (Female: 4.1 cm and 4.4 cm; Male: 4.9 cm and 5.2 cm) (Figure 3). There was significant difference between the distribution of length VH before and after CGL ($p < 0.0001$), suggesting a trend towards an increase in distance between the hyoid bone and the vertebrae following the procedure.

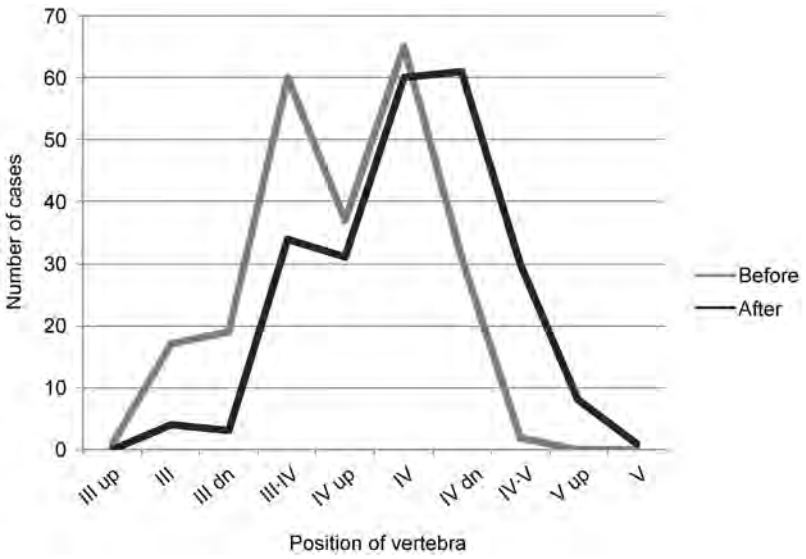

Figure 2. Distribution of the height of point V before and after CGL in the study sample (N = 232) ($p < 0.0001$).

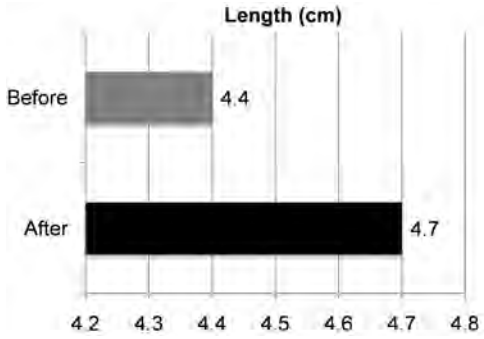

Figure 3. Mean length of segment VH before and after CGL in the study sample (N = 232) ($p < 0.0001$).

4. Discussion

Phylogenetically, the tongue appeared later. In fishes, the hyoid bones are in the inner margin of the mouth bones [6]. Fishes are able to eat and breathe with the composite of visceral skeleton. They don't need the tongue as mammals.

The tongue muscles are attached at the top of the hyoid bone in humans by the hyoglossal membrane. The tongue muscles are attached to the mandibular bone ventrally. The bottom edge of the hyoid bone the larynx is attached to the thyrohyoid ligament. In turn, the larynx is connected with the trachea, bronchi and lungs. These structures constitute a complex that is called the hyoid bone complex (HBC). The hyoid bone floats in the neck but and the mandibular bone is stable. As the hyoid bone is able to move upwards and downwards via the muscles that are attached to its top and bottom edge, the HBC is able to move upwards and downwards too [7]. As a result, the HBC in humans is pulled up and forward by the tongue. The CGL is a procedure that cuts the frontal bundles of genioglossus muscles and therefore the HBC can rotate downward and forward following CGL.

This study showed that the hyoid bone tended to move downwards of about half vertebra and about 0.3 cm ventrally following CGL. These outcomes may be interpreted in light of the observed improvements following CGL in individuals with ADEL. In fact, disappearance of symptoms and signs of ADEL following CGL (for example, improvement in respiratory function and increase in body temperature) may be related to downward and ventral movements of the hyoid bone that might accelerate respiration [8] [9] [10].

The improvement of symptoms of ADEL and the increase in respiratory function and body temperature observed after CGL may be related to downward and forward movements of the HBC that, in turn, may accelerate physiological functions and may, as such, have further implications in terms of not only respiration and swallowing but also circulation, metabolism, and immune function. The observed improvements are likely to be related to the function of the HBC, a complex structure that regulates respiration and various human functions such as drinking, laughing, singing, swallowing, vomiting, crying, and breathing.

It was reported that the hyoid bone of Homo Neanderthalensis is higher than that of Homo Sapiens [11] [12]. The stylohyoid bone of Homo Neanderthalensis was shorter than that of Homo Sapiens. Therefore the HBC of Homo Neanderthalensis might be shorter and it might have had more limited movements. Related to this, the metabolisms of Homo Neanderthalensis might have been lower than that of Homo Sapiens, possibly contributing to a higher mortality rate, thus suggesting that positional changes of the HBC, such as the ones induced by CGL and demonstrated by this study, may play a role in an evolutionary perspective.

5. Conclusions

The tongue pulled HBC upwards. The improvements in symptoms and signs of ADEL observed following CGL as downward and forward movement of the

hyoid bone could improve respiration and, in turn, contribute to improved physiological function.

There might exist a system that control respiration and swallowing as well as metabolism by the hyoid bone that is hyoid bone complex (HBC).

Consent

All included patients provided their written informed consent, and the study's protocol was approved by the research ethics committee of Yamato City Medical Association.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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Epidemiological Pattern of Breast Diseases among Females in the South-Western Region, Saudi Arabia

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Abstract

Background: Breast diseases cover several conditions. The majority of breast diseases are noncancerous. Some of these lesions are clinically unremarkable, which needed minimal intervention. However, some symptoms may be of clinical value and attract the attention of both the patient and the attending physician, especially when they become persistent. The study aimed to assess the prevalence, pattern, types, and clinical profile of breast diseases among females in the South-western region, during the period from 2018-2020. **Methods:** A retrospective record-based descriptive approach was used through reviewing medical records of all cases with breast disease attended King Khalid Hospital during the period from January 2018 to January 2020. Data extracted throng pre-structured questionnaire. **Results:** The study included 211 cases whose ages ranged from 18 to 58 years old with a mean age of 28.9 ± 12.8 years. Breast mass was the most recorded complaint (95.1%) followed with breast pain (32%), skin changes. Benign findings based on the final pathology report were recorded for more than two thirds of the cases, with the most common finding were fibroadenoma. Excisional biopsy and modified radical mastectomy were the most reported surgical interventions. **Conclusions:** In conclusion, the study revealed that the majority of the cases had benign breast disease (BBD), where fibroadenoma was the most frequent.

Keywords

Breast Disease, Females, Incidence, Pattern, Predictors, Epidemiologylesions, Biopsy

1. Background

Breast diseases make up several conditions. The majority of breast diseases are noncancerous [1] [2]. Breast lesions are mainly recorded among females. The most recorded cause of breast problems in females is a benign breast disease, which is 10 times more common than breast cancer in most countries [3]. Some of these lesions are clinically unremarkable, which needed minimal intervention. However, some symptoms may be of clinical value and attract the attention of both the patient and the attending physician, especially when they become persistent. The main item behind that concern is the possibility of the occurrence of breast cancer, which is usually asymptomatic in the early stages during which it is curable [4]. Benign breast diseases are mainly prevalent during the reproductive age as the incidence is common mainly in the second decade with realization on its peak at fourth and fifth life year's decade [5]. Benign breast diseases include many and variable histological patterns categorized into non-proliferative breast lesions, proliferative breast lesions without atypia, and proliferative breast lesions with atypia [6] besides fibroadenoma, fibrocystic change, and breast abscess are the most recorded benign lesions in the literature [7]. Certain types of benign breast lesions were recorded as a significant risk factor for developing breast cancer [8]. Women with benign proliferative or atypical breast lesions consume a two-fold risk of developing breast cancer in western populations [9].

The literatures covered the histopathological pattern of breast diseases with less attention for the clinical perspectives [10] [11] [12]. Surgical evaluation of the symptomatic patients by triple assessment, namely, clinical examination of the breast, mammography, and breast biopsy for definitive histological diagnosis is required in many patients [13].

The current study aimed to assess the prevalence, pattern, types, and clinical profile of breast diseases among females in the South-western region, during the period from 2018-2020.

2. Methodology

Retrospective record-based descriptive approach was used through reviewing medical records of all cases that were admitted and have breast disease (benign or malignant breast masses) in King Khalid Hospital during the period from January 2018 to January 2020. Records with missing data were included. Data extracted through pre-structured questionnaire including patient's bio-clinical data, initial clinical presentation of the breast disease, radiological assessment findings. Also, the final pathological findings were extracted besides the nature

of the assigned pathology. Data also included the type of surgery and post operative outcome.

3. Statistical Analysis

After data were extracted, it was revised, coded, and fed to statistical software IBM SPSS version 22 (SPSS, Inc. Chicago, IL). All statistical analysis was done using the two-tailed test. A P-value of less than 0.05 is considered being statistically significant. Descriptive analysis based on frequency and percent distribution was done for all variables, including demographic data, breast disease nature, and types, clinical presentation, and surgery outcome.

4. Results

The study included 211 cases whose ages ranged from 18 to 58 years old with a mean age of 28.9 ± 12.8 years. Exact of 59 (72%) of the females were married, and all of the sampled females started menstruation at the age of 18 years with regular menstrual cycle. Ten (67%) of the females are still menstruating. Only one (10%) of the study females had the first pregnancy before the age of 20 years and 10% above the age of 30 years. As for gravidity, 3 (10.3%) of the females had no pregnancy, while 31% had four pregnancies or more. Considering co-morbidity, diabetes mellitus was recorded among 5 (9.6%) of the females and 8 females (15.4%) had hypothyroidism (Table 1).

Table 2 demonstrates the determinants of having breast disease. Oral contraceptive pills were received by 8 (40%) of the study females, and 30.8% of them had hormonal therapy. A family history of breast disease, including breast cancer, was recorded for 4 females (23.5%). Only 8 (44.4%) of the females know about breast screening for early detection of breast lesions, while 23 females (85.2%) do breast examination regularly. Only one case recorded that she performs regular breast screening after the age of 40 years.

Concerning clinical data of recorded breast diseases (Table 3), breast mass was the most recorded complaint as recorded for 196 females (95.1%) followed with breast pain which was among 66 females (32%), skin changes (11; 5.3%), and nipple discharge (8; 3.9%). The symptoms = appeared for less than one month among 23.5% of the cases while lasted for more than 6 months among 52 females (39.4%). As for the affected side, the right side was dominant among 84 females (41.2%) of the cases while it was bilateral among 19 (9.3%). The breast mass measured less than 2 cm among 60 (37.5%) of the cases while it was more than 5 cm among 21 females (13.1%) of them. Margins of the breast mass were irregular among 46.2% of the cases, and the mass was soft among 59 of the patients (54.1%). Skin changes were recorded for 28 (22.2%) of the cases, and 53 cases (34.9%) had positive Axillary lymph nodes on physical examination.

Considering radiological and biopsy findings among the cases, Table 4 illustrates that the most recorded US finding was Fibrocystic disease (64; 49.6%) followed with cancer related features (29; 22.5%), breast abscess (8.5%), and fibroadenoma

Table 1. Personal and obstetric data for female patients with breast diseases in Najran, Southern Saudi Arabia.

Personal & obstetric data		No	%
Age in years	<20 years	17	8.1%
	20 - 30	42	19.9%
	30 - 40	65	30.8%
	40 - 50	75	35.5%
	50+	12	5.7%
Marital status (n = 82)	Single	19	23.2%
	Married	59	72.0%
	Divorced/widow	4	4.9%
Age of menarche	<18 years	20	100.0%
Regular menstrual cycle	Yes	22	100.0%
Age of menopause (n = 15)	Not at menopause	10	66.7%
	40 - 49 years	5	33.3%
Age of pregnancy (n = 10)	<20 years	1	10.0%
	20 - 30	8	80.0%
	30+	1	10.0%
No. of pregnancies (n = 29)	Nulligravida	3	10.3%
	1 - 3	17	58.6%
	4+	9	31.0%
Co-morbidity	None	10	19.2%
	DM	5	9.6%
	HTN	7	13.5%
	Hypothyroidism	8	15.4%
	Asthma	3	5.8%
	Others	8	15.4%
	Diabetic and HTN	11	21.2%

Table 2. Factors related to have breast disease among female patients in Najran, Southern Saudi Arabia.

Factors related to breast diseases		No	%
Oral contraceptive pills	No	12	60.0%
	Yes	8	40.0%
Hormonal therapy	No	9	69.2%
	Yes	4	30.8%
Family history of breast disease	No	43	76.8%
	Yes	13	23.2%
Family history of other cancers	No	13	76.5%
	Yes	4	23.5%
Know about breast screening	No	10	55.6%
	Yes	8	44.4%
Regular Breast Examination	No	4	14.8%
	Yes	23	85.2%
Who did the examination	By herself	12	52.2%
	By physician	11	47.8%
Regular breast screening after age 40	No	3	75.0%
	Yes	1	25.0%

Table 3. Clinical data of breast diseases among female patients in Najran, Southern Saudi Arabia.

Clinical data		No	%
Clinical signs & symptoms	Mass	196	95.1%
	Pain	66	32.0%
	Skin changes	11	5.3%
	Nipple changes	8	3.9%
	Swelling	4	1.9%
	Discharge	2	1.0%
	Others	2	1.0%
	Axillary mass	1	0.5%
Duration of symptoms	<1 month	31	23.5%
	1 - 2 ms	29	22.0%
	3 - 6 ms	20	15.2%
	>6 months	52	39.4%
Side of lesion	Right	84	41.2%
	Left	101	49.5%
	Bilateral	19	9.3%
Mass size	<2 cm	60	37.5%
	2 - 5 cm	79	49.4%
	>5 cm	21	13.1%
Margins of mass	Irregular	49	46.2%
	Regular	57	53.8%
Mass consistency	Soft	59	54.1%
	Hard	50	45.9%
Skin changes	No	98	77.8%
	Yes	28	22.2%
Nipple discharge	No	114	90.5%
	Yes	12	9.5%
Nipple retraction	No	89	84.8%
	Yes	16	15.2%
Nipple destruction	No	90	98.9%
	Yes	1	1.1%
Positive axillary lymph nodes	No	99	65.1%
	Yes	53	34.9%

Table 4. Radiological and biopsy findingsamongfemale patients with breast disease in Najran, Southern Saudi Arabia.

Radiological and biopsy findings		No	%
US findings	Fibrocystic disease	64	49.6%
	Cancer	29	22.5%
	Breast abscess	11	8.5%
	Fibroadenoma	10	7.8%
	Phyllodes	5	3.9%
	Unremarkable	2	1.6%
	Galactocele	1	0.8%
	Lactating adenoma	1	0.8%
	Mastitis	1	0.8%
	Mature adipose tissue with fibrous tissue	1	0.8%
	Antibioma	1	0.8%
Mammogram findings	Speculated mass	14	35.9%
	Microcalcification	10	25.6%
	Microcalcification, Speculated mass	9	23.0%
	Texture distortion	2	5.1%
	Fatty glandular breast parenchyma	1	2.6%
	Mixed fibro-fatty glandular breast prynchema	1	2.6%
	Multi cystic	1	2.6%
	Oil cyst	1	2.6%
MRI impression	Benign	21	41.2%
	Malignant	30	58.8%
Type of biopsy	Excisional	35	19.6%
	True cut	117	65.4%
	Fine needle aspiration cytology (FANC)	23	12.8%
	Incisional	3	1.7%
	Lumpectomy	1	.6%
Final pathology report	Benign	124	63.9%
	Malignant	70	36.1%
Nature of lesion	Fibroadenoma	66	42.9%
	Fibrocystic	29	18.8%
	Invasive ductal carcinoma (IDC)	29	18.8%
	Breast abscess	9	5.4%
	Atypical ductal hyperplasia	2	1.3%
	Mucinous carcinoma	2	1.3%
	Antibioma	1	0.6%

Continued

	Acute and chronic breast inflammation	1	0.6%
	Ductal ectasia	1	0.6%
	Ductal hyperplasia	1	0.6%
	Epidermoid cyst	1	0.6%
	Fibroepithelial lesion	1	0.6%
	Gynecomastia	1	0.6%
	Invasive mammillary carcinoma	1	0.6%
	Invasive papillary Ca	1	0.6%
	Lactating adenoma	1	0.6%
	Myofibroblastic tumor	1	0.6%
	Periductal & perilobular chronic inflammatory process	1	0.6%
	Phylloid with free margin less than 1 mm	1	0.6%
	Right axillary lipoma	1	0.6%
	Sebaceous cyst	1	0.6%
	Spidle cell tumor	1	0.6%
	Suggestive of tubular adenoma	1	0.6%
Ductal carcinoma *in situ*	No	18	56.3%
	Yes	14	43.8%

(10; 7.8%). Mammogram findings were also recorded with the most recorded one was speculated mass (14; 35.9%) followed with micro calcification (10; 25.6%), micro calcification with speculated mass (9; 23%), and texture distortion (2; 5.1%). MRI showed benign features among 21 cases (41.2%) of the cases. As for biopsy, 117 females (65.4%) of the cases undergone true cut biopsy followed with excisional biopsy (19.6%), and fine needle aspiration biopsy (12.8%). Benign findings based on final pathology report were diagnosed for 124 (63.9%) of the cases with the most common finding was fibroadenoma (66; 42.9%) followed with the fibrocystic disease (29; 18.8%), IDC (29; 18.8%) and breast abscess (9; 5.4%). Ductal carcinoma *in situ* was recorded for 43.8% of the cases.

Table 5 shows that Estrogens/Progestin Receptors were positive among 28 females (73.7%) of the cases and Human epidermal growth factor receptor 2 (HER2) was positive for 11 cases (30.6%) of the cases while Antigen Ki-67 was more than 12% among 10 cases (76.9%).

Regarding surgery and clinical outcome (Table 6), excisional Biopsy was the most surgical procedure (53; 41.1%) followed with modified radical mastectomy (28; 21.7%), lumpectomy (23; 11.6%) while 17.8% of the cases were not operated.

5. Discussion

Researchers reported wide variability in the frequency and distribution of breast

Table 5. Laboratory findings among female patients with breast disease in Najran, Southern Saudi Arabia.

Laboratory findings		No	%
Estrogens/progestin receptors	Negative	10	26.3%
	Positive	28	73.7%
Human epidermal growth factor receptor 2 (HER2)	Negative	25	69.4%
	Positive	11	30.6%
Antigen Ki-67	Less than 12%	3	23.1%
	More than 12%	10	76.9%

Table 6. Surgery data forfemale patients with breast disease in Najran, Southern Saudi Arabia.

Surgery data	No	%
Type of surgery		
Excisional biopsy	53	41.1%
Modified radical mastectomy	28	21.7%
Not operated	23	17.8%
Lumpectomy	15	11.6%
Incision and drainage	4	3.1%
Drainage	2	1.6%
LAMA	1	0.8%
Not mentioned	1	0.8%
Simple mastectomy	1	0.8%
Drainage	1	0.8%
Surgery complications		
None	87	83.7%
Seroma	9	8.7%
Wound infection	5	4.8%
Lymphedema	2	1.9%
Echymosis around the wound	1	1.0%

ailments across the world [14] [15] [16]. Breast diseases more recorded among women than men, the prevalence rate in males ranging from 0% to 5.8% in most series, and the majority of male breast lesions are benign with gynaecomastia [17]. As for females, the pattern of pathology had high variability based on age and geographical location. Benign lesions are prevalent at all ages, constituting 48.9% to 57% with the main age of occurrence below the 30 years [18] [19].

Benign breast lesions are more presented than cancer [20] [21] [22]. Benign breast diseases, however, formed a heterogeneous group of disorders, including developmental abnormalities, epithelial and stromal proliferation, inflammatory lesions, and neoplasms [23]. Breast cancer incidence increased from 12.7 million

in 2008 to 14.1 million in 2012, and this trend is predicted to increase in the future [24] [25]. It was estimated that worldwide over 508,000 women died in 2011 due to breast cancer [26]. Breast cancer survival rates vary greatly worldwide, ranging from 80% or over in North America, Sweden, and Japan to around 60% in middle-income countries and below 40% in low-income countries [27].

The current study aimed to assess the pattern of different breast diseases recorded for females in the south-western region, Saudi Arabia, during the period from January 2018 to January 2020. Also, the study aimed to assess the type of diagnostic and management methods with the clinical outcome. The study revealed that the majority of cases aged below 30 years and were married and still menstruating. Many risk factors for having breast diseases were recorded, including receiving oral contraceptive pills, hormonal therapy, positive family history, and irregular breast examination, especially at the age above 40 years. Breast mass was the most frequent complaint with breast pain with its size ranging from 2 - 5 cm in most cases and irregular margins in nearly half of the cases associated with Axillary lymph nodes in about 1 out of each 3 cases. Final pathology after a biopsy revealed that lesions were benign among two-thirds of the cases especially fibroadenoma, which is the most benign breast lesion. Regarding surgical intervention, modified radical mastectomy was needed for 1 out of each 5 cases, while only 11% of the cases undergone lumpectomy as the lesions were benign. Most of the cases had no post-operative complications less than 10% had seroma.

Generally, these findings were consistent with most of the literature. A study was conducted to assess benign breast lesions (BBD) in an African population [28] and recorded an increasing incidence of these benign lesions. The overall mean age for BBD was 27.5 years, SD ± 11.3, with an age range of 9 - 84 years and a peak age occurrence in the third decade. The single most common lesion was fibroadenoma accounting for 43.1% of cases, followed by fibrocystic change (23.8%). A second study was conducted by Ayoade BA *et al*. 2012 [29] to Clinical Features and Pattern of Presentation of Breast Diseases in Surgical Outpatient Clinic of a Suburban Tertiary Hospital in South-West, Nigeria. The researchers reported that the commonest symptoms were breast lump in 91.7% patients, and breast pain in 23.1% patients. Forty four patients (36.3%) had a malignant disease, seventy patients (57.8%) had benign breast diseases, and seven were normal. Fifty nine of the 70 benign diseases were fibroadenoma. In India, Hatim K *et al*. 2017 [30] conducted a research to assess Patterns and prevalence of benign breast disease in Western India. The reported that the commonest benign breast lesion was fibroadenoma (77.62%), followed by fibrocystic disease (4.3%) and gynaecomastia (4.3%).

Locally, Samir S *et al*. 1995 conducted a study to the spectrum of breast diseases in Saudi females [31]. The researchers reported that fibroadenoma was the most common lesion encountered 30.7%, followed by fibrocystic condition (21.1%), carcinoma (14.9%), acute mastitis (7.2%), duct ectasia (4.9%), lactational ade-

noma (4.8%), intraductal papilloma (2.6%), galactocele (2.4%) and several less frequent lesions. A second study was conducted to assess histopathological patterns of breast lesions in Northern Saudi Arabia, 2017 [32]. The study revealed that 23.2% of the lesions were ductal carcinoma, 4.4% were lobular carcinoma, 1.9% were mixed tumours, while 64.6% were fibroadenoma.

When local breast disease distribution patterns are known, skills related to diagnosis and management can be made with a sound degree of certainty. In addition, resource allocation and planning can be better allied. This is true mainly in low income countries where a large population of individuals may not afford diagnostic and management costs.

6. Conclusion and Recommendations

In conclusion, benign breast diseases (BBD) were the most recorded among Najran females, especially fibroadenoma, with low post surgical complications. The better reporting system should be initiated, and well training for the physicians is mandatory to improve the quality of available data, and research based on these data and achieve better and more realistic conclusions for planning and resource allocation.

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics and Research Committee of the College of Medicine of Najran University.

Limitations of the Study

Irrespective of the variety of collected clinical data in the current study, but there were two main limitations. The first is the sample size, which was due to reviewing only 2 years' cases, but this was due to a poor recording system, which somewhat primitive. The second was the high missing rate in the data for the cases, which also related to the primitive recording system and lack of physician's experience regarding these cases management guidelines.

Authors' Contributions

Conceptualization, Abdulrahman Manaa sultan Alamri; methodology, Abdulrahman Manaa sultan Alamri, Saeed Ali Alsareii; data curation, Hajr Hassan Al-Wadei, Awad Mohammed Al-Qahtani, Salem Ali Alatef Sultan, Sara Ali Alshamrani, Ahmed Hamzah Almakrami, Abdullah Ahmed Daiel; writing original draft preparation, Ahlam Yahya Alyami, Ashwaq Mousa Hommadi, Yagoub Mohammed Tahir Ali; funding acquisition, All participants. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Full Length Spine CT and MRI

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Abstract

Spine is a complex and long structure in the human body. Visualization of the spine is essential to treat and manage spine disease and commonly requires further imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI). In most clinical fields, spine CT and MRI examinations are focused on the region of interest. However, spine is composed of cervical, thoracic, lumbar, sacrum and coccyx and sometimes demands examination of entire structure as well as regional spine depending on disease, patient's state and physician's decision. This review considers the available literature to describe when and how full length spine evaluation by CT and MRI is applied according to each spinal disease such as spinal trauma, deformity, infection, axial spondyloarthropathy and metastatic tumor.

Keywords

Full Length Spine CT, Full Length Spine MRI, Trauma, Deformity, Infection, Axial Spondyloarthropathy, Metastasis

1. Introduction

There are multiple imaging modalities to evaluate the spine. The type of imaging tool for the spine depends on the type of disease, the amount of radiation hazard, contraindications and any allergy to contrast.

Among the multiple imaging strategies, computed tomography (CT) is a reliable method to evaluate the spine and has better sensitivity for bony abnormality than radiography or magnetic resonance imaging (MRI) [1]. In the past, the time factor limited the use of CT. However, owing to the development of fast spiral CT and multidirectional CT (MDCT), the whole spine can be examined in very short time [2]. CT is less sensitive to patient's movement than MRI [3]. Now, it is widely used to assess trauma, deformity, metastasis, pre and post-operative

patients.

MRI is one of the most sensitive and specific modalities to visualize the spine. Furthermore, there are no risks of radiation hazard. It provides information about the spinal neural structures including cord, cauda equina and roots, the vertebral bodies and intervertebral disc. Multiple sequences can be obtained in different planes and each sequence helps in evaluating various changes of the spine. The most commonly using sequences include sagittal and axial T1 and T2, and a sagittal STIR (short tau inversion recovery). Post-contrast T1 weighted images are useful for evaluating spinal infections, postoperative complications, vascular malformation, primary spinal tumors, and metastases. STIR sequences are beneficial in assessing marrow edema, which demonstrates an increased signal on these sequences [4].

In most cases, spine CT and MR images are taken focusing on the region of interest where the pathology exists. However, full length spine examination is more helpful in detailed evaluation and early detection of disease and trauma being able to involve the entire spine (**Figure 1**). Therefore, the aim of this review was to describe when and how full length spine evaluation through CT and MRI is applied.

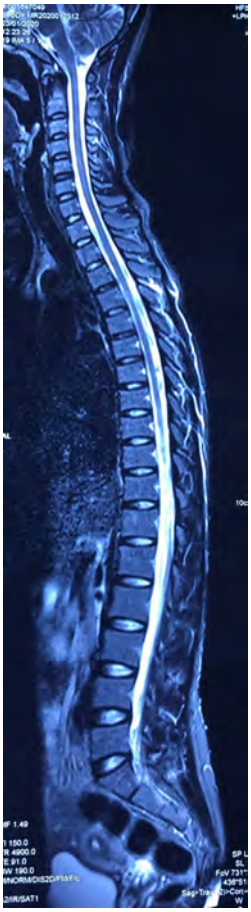

Figure 1. Normal sagittal T2 weighted magnetic resonance image of full length spine.

2. Methodology

A literature review using PubMed and Google Scholar was performed. To identify relevant studies, search was conducted on studies in English published between 2000 and February, 2020 using various combinations of the following key words: "full length spine", "entire spine", "whole spine", "imaging", "computed tomography". "magnetic resonance imaging". Based on this search, 127 studies were initially detected. After excluding articles about rare disease, technical aspect of CT and MRI, measurement and imaging protocol, 56 articles were selected. After an internal peer review with all the authors of this article and a supplementary search that involved bibliographic screening and citation tracking associated with individual spine pathology, 24 articles of interest were finally selected (Figure 2). Those articles are categorized as following sections: "Trauma", "Deformity", "Infection", "Axial spondyloarthropathy" and "Spinal metastasis".

3. Contributions of Full Length Spine CT and MRI

3.1. Trauma

When trauma to the spine occurs, according to the findings of physical examination and simple radiography, targeted CT and/or MRI is usually performed on the suspected spine as a further imaging work up. However, if the patient sustains

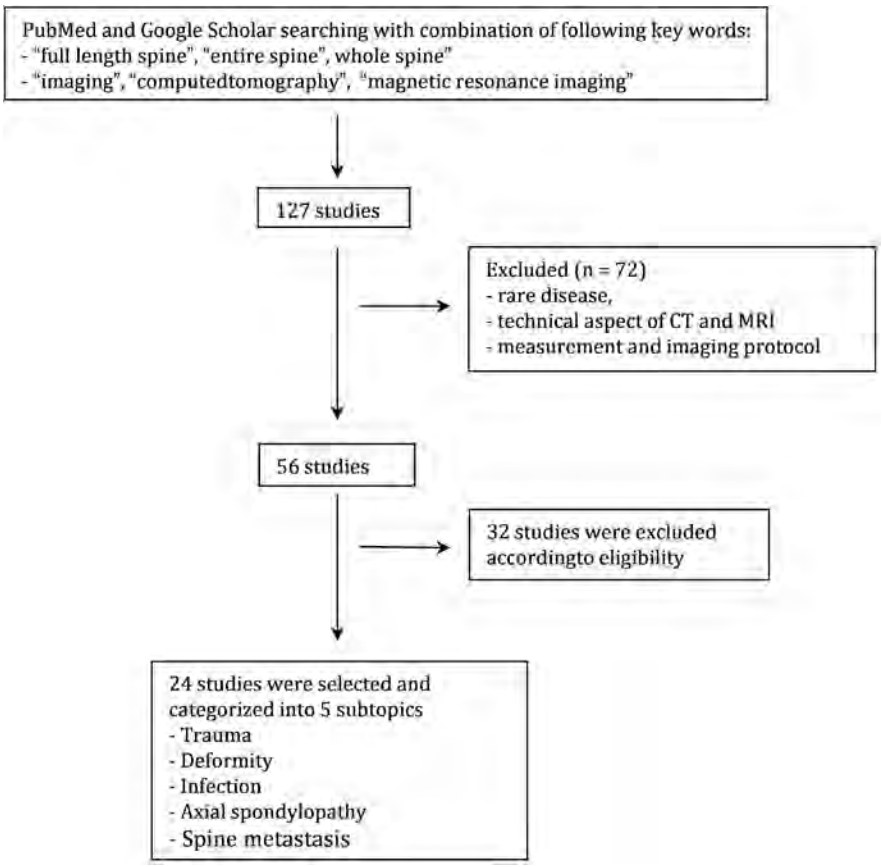

Figure 2. Methods of relavant study selection.

high energy trauma or multiple trauma, those spine imaging can be insufficient. In high energy or multiple trauma patients, after the patient's condition is stabilized, unforeseen injuries to the spine should be delineated accurately because the symptoms of a spinal fracture may be masked by concomitant injuries, patient's state of consciousness (**Figure 3**), spinal cord injury or shock [5] [6]. In these situations, it requires full length spine evaluations with CT and/or MRI rather than simple radiographs, targeted CT or MRI.

In the patients with high energy blunt trauma, Deunk *et al.* reported that the non-targeted CT detected 8.2% more spine injuries unrevealed by simple radiographs than the targeted CT [7]. In a prospective study, spinal fractures were prevalent in 30.2% of cases of high-energy trauma. Among patients with a cervical fracture, 37.5% had a fracture that unrevealed on radiographs of the cervical spine but were first diagnosed by a CT scan. Patients with a thoracolumbar fracture did not have clinical symptoms of a fracture in 14%, but had a fracture revealed by CT. Therefore, Takami *et al.* postulated that the full length spine CT should be taken to evaluate high energy trauma patients [6].

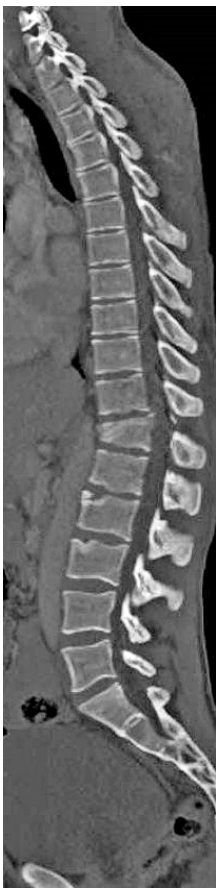

Figure 3. Sagittal full length spine computed tomography image showing Denis B type T12 fracture with widened interspinous space. A 29-year-old man sustained an injury from vehicle-pedestrian accident. The patient was unconscious in the emergency room.

Using full length MRI in spine trauma patients remains controversial. Atsina *et al*. described that full length MRI is not usually recommended in patients with spine trauma. When the full length MRI is performed in patients with blunt trauma after full length spinal CT, most additional spinal injuries detected on MRI were bone contusions and mild compression fractures. These additional spinal diagnoses are unlikely to change the management or treatment [8]. Therefore, the targeted MRI is more useful in patients with high energy blunt trauma. However, Kanna *et al*. reported that full length sagittal T2 weighted images help diagnose multi-level non-contiguous spinal injuries in spinal trauma patients and can be performed in approximately 4 minutes without radiation hazard [9].

3.2. Deformity

Spinal deformity is a complex disease with different three-dimensional abnormalities that often involve the entire spine [10]. In cases with a complex or severe spinal deformity, full length spine CT should be taken of the entire area of spine including the involvement segment of the spine deformity because radiography alone is insufficient to visualize complicated abnormalities of spinal deformity [11]. Furthermore, in comparison with radiography, full length spine CT scanning provides additional information about extent of rotation of the spine, segmentation defects, three-dimensional reconstruction and detection of bony spur in diastomatomyelia and associated congenital anomalies of ribs, scapula, and pelvis [12].

Full length spine CT is also useful for surgical planning. Current surgical techniques for deformity correction is making surgeons capable of correcting the coronal, sagittal and rotational aspects by the 3D assessment of spinal deformity [13]. Multiplanar reconstruction of the axial CT images is important, especially when trying to understand the anatomy of deformity and allows visualizing levels where osteotomies or resections are planned. The CT scan allows surgeons to understand the pedicle anatomy including the width, depth, and trajectory of the pedicles. During a deformity surgery, caution must be placed when placing screws in the pedicles, especially on the concave side of curvature where pedicles are extremely narrow and the spinal cord is vulnerable to the iatrogenic injuries due to restricted epidural space [14]. Preoperative full length spine CT in this context has been recommended because of the high probability of the presence of narrow pedicles. It also allows surgeons to identify pseudarthroses if the patients underwent previous fusion surgery. Postoperative full length spine CT is considered for patients with a new neurologic deficit after surgery. Postoperative CT helps to find the malposition of pedicle screws that develops postoperative neurological symptoms [15].

Full length spine MRI usually is obtained in patients with spinal deformity who develop neurologic symptoms or are planned to be operated. MRI provides good visualization of neurological structures, and helps determine appropriate levels of surgery, and can assist in the evaluation of disc degeneration when fu-

sion extension is considered [16]. MR imaging is also essential for the patients with an unusual curve pattern and for those patients who should be assessed on the neural axis. It is important to understand whether syrinx, tethering of the spinal cord, intramedullary tumors or Chiari malformations are present. The presence of those intraspinal anomalies without neurological findings in idiopathic scoliosis has been estimated between 4% and 26% and associated with neurological complications resulted from correction surgery [17]. Stenosis or cord impingement secondary to severe deformity can be visualized by full length MRI. In kyphoscoliosis, the level of cord compression should be identified preoperatively and always on the concave pedicle. Those compressions may require a resection of that pedicle with the remaining body during deformity surgery [11] [15].

3.3. Infection

The diagnosis of spinal infection can be difficult, and even delayed because clinical manifestations can be varied and equivocal. Clinical physicians have relied heavily on imaging modalities due to difficulty in detecting spinal infection. Among imaging modalities, MRI plays a pivotal role in the diagnosis and management of spinal infection with a sensitivity, specificity, and accuracy above 90% and the ability to evaluate the spine neural structures [18].

If disease process of spinal infection is strictly localized, it is not necessary that the entire spine is examined. However, identification of one infected site in the spine cannot rule out the infection in multiple region of the spine. The areas adjacent to anterolateral vertebral endplates has abundant vascular supply and are most common regions where pyogenic, tuberculous, and other spinal infections occur primarily. Associated edema is commonly pronounced and affects the entire vertebral body and intervertebral disc [19]. If there is epidural involvement associated with neurologic symptoms, it is recommended to take full length spine images under these circumstances because epidural abscesses can spread extensively [20]. Among spinal infections caused by various organisms, mycobacterium tuberculosis are notorious for multifocality, and subligamentous spread of infection to multiple levels is one of the imaging hallmarks of the disease. The infection typically starts in the anterior aspect of the vertebral body adjacent to the intervertebral disc and then spreads to the vertebral bodies under the anterior longitudinal ligaments involving multiple adjacent vertebrae. Through valveless vein system, it can also make non-contiguous lesion in another spine levels other than where the infection started [21].

The incidence of multifocal spinal infection has varied from around 4% to 23%. Mann *et al.* evaluated 24 patients with spondylitis and found 21 patients had single level infections**,** while 1 (4%) had multi-segmental infection [22]. Ledermann *et al.* found 7 (16%) out of 44 of patients with disc infection had involvement at several spinal levels [23]. Cox *et al.* reported that 82 patients with single-level infection performed full length MRI examination and 19 (23%) pa-

tients had additional non-continuous sites of infection. Remote levels of spondylodiscitis were also present in 11 patients (13%) [20]. In cases of multifocal spinal tuberculosis, the reported incidence is variable, ranging from 1.1% to 71.4%. Kaila *et al*. reported a very high incidence of 71.4% [24], but their data is likely overestimated due to the small number of cases presented. According to a retrospective study, 47 (25.1%) of 187 patients with spinal tuberculosis had multifocal spinal tuberculosis [25].

In the spinal infection, full length spine MRI can be used as a diagnostic screening test to detect multifocal lesions even if patients do not have clinical symptoms and provides early detection of spinal infection preventing progression of osteomyelitis. In regard to the management and treatment of spinal infection, the detection of an epidural abscess or other infective lesions remote from the site of known uncomplicated spinal infection will likely result in a change of management and treatment from conservative to surgical [26].

3.4. Axial Spondyloarthropathy

In the management and treatment of axial-spondyloarthropathy (SpA), the benefit of early detection before structural damage begins is of importance. However, clinical findings of early axial-SpA are not specific and assessment can be challenging as the structural components of the spine is not easy to assess due to the deep location. Furthermore, current imaging modalities such as radiographs, CT, and bone scintigraphy have shown limitations for use in early detection of axial-SpA. Both CT and bone scintigraphy expose the patient to high levels of ionizing radiation; bone scintigraphy showed low specificity and CT cannot detect until structural bone changes have progressed [27]. On the other hand, MRI has shown high sensitivity and specificity of imaging tools to pre-structural early inflammatory changes taking the presentation of bone marrow edema adjacent to joints and discs, entheses, and ligaments [28].

Although MRI of the sacroiliac (SI) joints has traditionally been used in suspected axial-SpA, this may be inadequate. Inflammatory lesions in the spine have been found most commonly in the thoracic spine besides SI joints. Full length spine MRI highlighted the frequent involvement of the thoracic spine, with emphasis on the most lateral sagittal aspects of vertebral endplates and costovertebral junctions. About 23% of ankylosing spondylitis patients with clinically active disease only have inflammatory spinal lesions and no evidence of active inflammatory sacroiliitis, even in very early disease. Cervical spine involvement is also common in ankylosing spondylitis. For these reasons, full length spine MRI of the whole spine including SI joints is an essential tool in the diagnosis, management and prognosis of axial-SpA [29].

Full length spine MRI has been integrated into recommendations for the staging and therapeutic response evaluation in ankylosing spondylitis. It has been used to evaluate the therapeutic response showing the transition from active enthesitis with bone marrow edema to quiescent fatty infiltration of the

bone marrow [30].

3.5. Spine Metastasis

Plain radiography, CT, and MRI comprise the core imaging modalities for patients with vertebral metastases. CT provides cross sectional images, allows for entire spine imaging, is suitable to visualize cortical and trabecular bone, and is more sensitive than conventional radiography. CT scans can detect a bony metastatic lesion up to 6 months earlier than an X-ray. However, when compared to MRI, Buhmann *et al.* found the diagnostic accuracy of MRI (98.7%) to be significantly superior to MDCT (88.8%) for the detection of osseous metastases. Sensitivity was significantly lower for MDCT (66.2%) than for MRI (98.5%) [31]. Moreover, cortical destruction may be difficult to be detected in CT exam when osteoporosis or degenerative changes occur. There is also an inherent associated risk of radiation exposure. Changes in bone-marrow are fundamental to the sensitivity of MRI in the detection of sites of skeletal metastases in the spine. The combination of unenhanced T1-weighted spin echo and STIR sequences have shown to be most useful for the detection of bone marrow abnormalities and are able to discriminate benign from malignant bone marrow changes [32].

Therefore, contrast-enhanced MRI of the entire spinal axis is the current standard for the diagnosis and evaluation of spinal column metastases. If the MRI examination is limited to the spine region of interest, and bone marrow metastases outside the image volume can be missed. Full length spine MRI is often imperative to visualize multiple levels of spinal involvement with asymptomatic disease or the large amount of bone destruction on plain radiography. Full length spine MRI including pelvis has been used to detect bone metastases and hematologic malignancies. In prostate cancer, full length spine MRI including pelvis is highly sensitive for the detection of metastases in the axial skeleton (**Figure 4**). In multiple myeloma, the axial skeleton approach with entire spine and pelvis is recommended in the staging system [33].

After radiation therapy, MRI appears to be a powerful tool for differentiating post-therapeutic changes from tumor recurrence. CT is not used routinely during follow-up, but may be necessary for surveying osteoblastic, osteolytic or mixed lesions. MRI is also useful for evaluation of paravertebral masses and epidural extension [34].

4. Summary

Full length spine CT and MRI plays a significant role in diagnosis and management in spinal trauma and disease. Full length spine CT helps diagnose unforeseen injuries to the spine that may be missed due to concomitant or patient conditions in high energy and multiple trauma patients. In the patient with complex or severe deformity, full length spine CT facilitates to analyze deformed anatomy and establish preoperative plan. Neurological structures and intraspinal anomalies can be visualized by full length MRI, thereby can prevent

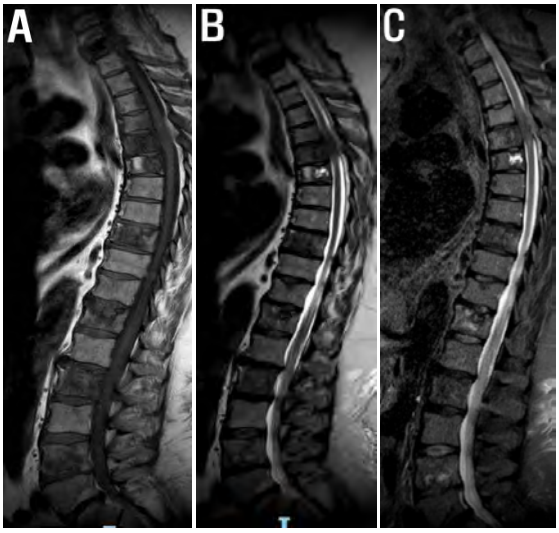

Figure 4. Sagittal full length spine T1 weighted (A), T2 weighted (B) and STIR (C) MR images shows multiple spinal metastasis originating from a prostate cancer.

neurological complications after deformity surgery. In multifocal spinal infections, entire spine should be visualized through MRI to evaluate the extension of infection and plan for the treatment. Axial SpA can be detected in early disease course with help of full spine MRI including SI joints. Metastasis to the spine is probably the most common indication for full length imaging as it allows evaluation by bone marrow change through whole spine MRI, especially in metastasis from prostate cancer and multiple myeloma. Due to the development of technology, taking time for CT and MRI of entire spine is being shorter and the resolution is increasing, and in particular, in the case of CT, the radiation exposure is gradually reducing. These changes will expand the indications for examining full length and help to diagnose, treat and follow up the patients with spine trauma and disease.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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COVID-19 Coronavirus: Is Infection along with *Mycoplasma* or Other Bacteria Linked to Progression to a Lethal Outcome?

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Abstract

Most patients with COVID-19 disease caused by the SARS-CoV-2 virus recover from this infection, but a significant fraction progress to a fatal outcome. As with some other RNA viruses, co-infection or activation of latent bacterial infections along with pre-existing health conditions in COVID-19 disease may be important in determining a fatal disease course. *Mycoplasma* spp. (*M. pneumonaie*, *M. fermentans*, etc.) have been routinely found as co-infections in a wide number of clinical conditions, and in some cases this has progressed to a fatal disease. Although preliminary, *Mycoplasma pneumoniae* has been identified in COVID-19 disease, and the severity of some signs and symptoms in progressive COVID-19 patients could be due, in part, to *Mycoplasma* or other bacterial infections. Moreover, the presence of pathogenic *Mycoplasma* species or other pathogenic bacteria in COVID-19 disease may confer a perfect storm of cytokine and hemodynamic dysfunction, autoimmune activation, mitochondrial dysfunction and other complications that together cannot be easily corrected in patients with pre-existing health conditions. The positive responses of only some COVID-19 patients to antibiotic and anti-malaria therapy could have been the result of suppression of *Mycoplasma* species and other bacterial co-infections in subsets of patients. Thus it may be useful to use molecular tests to determine the presence of pathogenic *Mycoplasma* species and other pathogenic bacteria that are commonly found in atypical pneumonia in all hospitalized COVID-19 patients, and when positive results are obtained, these patients should treated accordingly in order to improve clinical responses and patient outcomes.

Keywords

Pathogenic *Mycoplasma*, SARS-CoV-2 Virus, COVID-19 Disease, Acute

Respiratory Distress Syndrome, Co-Infection, Pneumonia, Lethal Infection, Mitochondria, Cytokines, Anti-Microbial Therapy, Antibiotics, Anti-Malarial Therapy, Virus, Bacteria

1. Introduction

The appearance of an outbreak of unexplainable pneumonia in Hubei province, China in 2019 revealed that a new coronavirus named 2019-nCoV (renamed SARS-CoV-2 coronavirus) was the cause [1] [2]. Patients presented with respiratory and other symptoms, such as cough, fever, and lung damage, along with fatigue, myalgia, dyspnoea, arthralgia, diarrhea, vomiting, headache, among other symptoms [2] [3].

Outcomes of COVID-19 vary from mild, self-limiting disease with respiratory symptoms to more severe manifestations and death [3] [4]. Patients with COVID-19 that progressed to death generally were older and had other underlying health conditions, such as hypertension, cardiovascular disease, chronic obstructive pulmonary disease, diabetes, chronic kidney disease, malignancy, or other conditions [4] [5]. The severe complications associated with non-survival from COVID-19 were primarily acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, coagulation dysfunction and multiple organ failure [5] [6] [7]. Among the most common organ failures were lung, heart and kidney [7]. Rarely mentioned in these articles was the possibility that other bacterial or viral co-infections could be contributing to either the pathogenesis of SARS-CoV-2 or to the lethal phase of the disease.

This contribution will focus on a possible role for intracellular bacterial infections, such as *Mycoplasma* species and other possible intracellular bacteria (*Chlamydia pneumoniae*, among other possible infections), in the progression and non-survival of COVID-19 patients. Such infections, if present, could contribute to the lethality of the SARS-CoV-2 virus in COVID-19 patients.

2. *Mycoplasma* Species

One of the most commonly found co-infections in a variety of chronic health conditions and diseases are various pathogenic *Mycoplasma* species [8] [9] [10]. Pathogenic *Mycoplasma* species infections are usually community-acquired infections that are non-fatal, but some patients can progress to a fulminant, systemic disease that results in death [11] [12].

Mycoplasmal infections are often associated with other bacterial and viral infections [8] [9] [10] [13] [14] [15], and the presence of multiple *Mycoplasma* species (and other bacteria and viruses) has been statistically associated with more severe signs and symptoms in chronic illnesses [14]. An example is tick-borne Lyme disease where several co-infections are involved in causing complex clinical presentations, and in this example Mycoplasma was usually the most

common Lyme disease co-infection found with *Borrelia* species and other infections [15] [16]. Also, mycoplasma infections are often found in community-acquired pneumonia as co-infections with influenza and other infections [17] [18] [19] [20] [21]. Co-infections of mycoplasma have been found previously in patients with SARS virus infections [22].

Pathogenic mycoplasmas are often found as respiratory tract infections that induce airway inflammation and bronchial hyper-responsiveness (BHR) [23]. For example, the induction of *M. pneumoniae*-specific IgE and IgA is likely to play an important role in exacerbating BHR and asthma. Indeed, elevations of IgE antibodies specific to *M. pneumoniae* have been detected in the serum of patients with *M. pneumoniae*-induced pneumonia. The serum levels of specific IgE and IgA followed infection with *M. pneumoniae,* and this was especially true in patients with pre-existing asthma-BHR [24]. *M. pneumoniae* was found in 24.7% of patients with asthma-BHR but in only 5.7% of control subjects [25].

Mycoplasma pneumoniae has recently been identified in COVID-19 disease [26]. This communication [26], along with a different case report [27], suggested that *Mycoplasma* should be considered as a possible co-infection in progressive COVID-19 disease. In a separate study with 138 patients with COVID-19, 26.5% of COVID-19 patients were found to have *Mycoplasma* species infections [28]. This percentage may be low due to the insensitivity of the testing procedures used [28]. Other related bacteria, such as *Chlamydia pneumoniae* and other *Chlamydia* species were also found at similar levels, but it was not clear from this contribution whether this occurred in the same patients that were positive for mycoplasma or in different patients [28]. COVID-19 patients are rarely examined for intracellular bacterial infections like mycoplasma [2] [3] [4] [5] [6]. In the cases where bacterial infections have been considered, *Klebsiella pneumoniae*, *Aspergillus flavus*, *A. fumigatus*, extended spectrum β-lactamase-positive (ESBL) *K. pneumoniae*, ESBL-positive *Pseudomonas aeruginosa*, and ESBL-negative *Serratia marcescens*, and *Candida albicans* have been found, usually in individual patients. When these bacterial infections were found in COVID-19 patients, they were considered hospital-acquired and unrelated to patient mortality [7].

3. Oxygen Deprivation and Mitochondria

The usual clinical course for patients with fatal COVID-19 disease has been found to be progression to critical ARDS requiring oxygen and mechanical ventilation [1] [2] [3] [7]. In the severe cases of SARS-CoV-2 infections the most significant difference between surviving patients and non-surviving patients was the ratio of partial pressure of oxygen (PaO_2) to fraction of inspired oxygen (FiO_2, from arterial blood gas analysis)or acute onset hypoxemia [7]. The ratios of PaO_2 to FiO_2 were found to be significantly lower in non-survivors, consistent with severe ARDS [7] [29]. Even with extracorporeal membrane oxygenation [7] or inhaled pulmonary vasodilators [20], critical COVID-19 patients failed to survive.

This suggests that oxygen is not being taken in, utilized effectively and passed into the circulation by lung tissues, possibly because cells infected with SARS-CoV-2 virus and other intracellular bacterial co-infections have lost their abilities to maintain proper cellular and circulatory oxygen levels.

In cells oxygen is utilized by mitochondria to produce high-energy molecules through oxidative phosphorylation. Mitochondria are also required for other critical functions, such as regulation of ion and redox homeostasis, biosynthesis of lipid and other metabolites, innate immunity, autophagy, cell signaling, regulation of cell death, and other cellular functions [30] [31]. Mitochondria can be affected negatively by several types of infections, including coronaviruses (see below) and mycoplasmas [32] [33] [34] [35]. For example, *Mycoplasma pneumoniae* infection results in the excess production of Reactive Oxygen Species (ROS) that can damage mitochondrial membranes and mitochondrial DNA. ROS have also been found to interfere with mitochondrial metabolism and stress responses in human lung cells [35]. Infections like mycoplasma also steal important mitochondrial metabolites that are needed for mitochondrial function and produce toxic molecules that damage mitochondria and cells and affect mitochondrial function (see below).

In COVID-19 patients compromised oxygen exchange in the air sacs of the lungs could be the cause of dyspnea or hypoxia [36]. However, the problem is likely to be more systemic, involving widespread mitochondrial dysfunction in endothelial cells and in various tissues and organs affected by SARS-CoV-2 and other co-infections. Possible co-infections include various mycoplasmas and other intracellular bacteria that can cause mitochondrial dysfunction [32]-[37]. In addition to lungs, organ damage can also occur in other tissues, such as heart and kidney, and cause their failure [2] [3] [7].

4. Suppression of Host and Mitochondrial Responses

Mitochondria have an impact on the pathogenesis of many common diseases and disorders, including neurodegenerative diseases, metabolic diseases, cardiovascular diseases, fatiguing illnesses, among others, and importantly for this discussion, infectious diseases [31] [32] [33] [37] [38]. Some infections alter mitochondrial dynamics and promote pathogenesis that benefits the infectious process [33]. For example, SARS coronaviruses interfere with mitochondrial mitophagy and innate immunity against infections [34]. This will be discussed in more detail in the next section.

In acute COVID-19 cases with severe respiratory complications (ARDS) the patients who died had severe massive alveolar damage and progressive respiratory failure [7] [36], even in cases where antiviral and corticosteroid therapies were given in an effort to attenuate pulmonary inflammation [36]. Lymphocytopenia has been a common finding in COVID-19 patients [29], but this finding has not been useful in identifying whether a patient will survive or not [7]. When mononuclear cells were examined in COVID-19 patients, their status was

concluded to be hyper-activated, with high proportions of CD4, CD8 and CD38 cells, increased numbers of proinflammatory cells, and high concentrations of cytotoxic granules inside cells [36].

Pathogenic mycoplasmas, such as *M. pneumoniae*, are known to cause community-acquired atypical pneumonias with immunological manifestations [12] [24] [39]. These infections are typified by inflammatory reactions and immune suppression [12] [24] [40] [41] [42]. However, mycoplasmas do not possess typical bacterial cell walls that contain inflammation-inducing endotoxins, such as lipopolysaccharides [12] [41]. Instead, mycoplasmas contain lipoproteins that can induce inflammatory responses through Toll-like (TLR) and other receptors, and they induce release of pro-inflammatory cytokines that contribute to the clinical problem [41] [42] [43]. This will be discussed further in Section 7.

Since the populous has not been exposed previously to the SARS-CoV-2 virus, they generally do not possess immunity to this infection and thus adaptive immune responses are non-existent [44]. However, based on findings with other coronaviruses, such as SARS-CoV, host innate immune response systems that utilize pattern recognition and TLR receptors will likely be involved in initial responses [45]. Even when the adaptive immune responses are initiated, involving various T cell linages and B cell production of antibodies, SARS-CoV-2 may initiate immune suppression by inducing apoptosis of T cells. This type of B-cell humoral immunity is thought to be important in combating infections of SARS-CoV-2 [44].

5. Blood and Coagulation Disturbances

Patients with COVID-19 disease and atypical pneumonia tend to show blood disturbances, such as leukoctyopenia, lymphocytopenia and thrombocytopenia, along with elevated levels of aspartate aminotransferase, alanine aminotransferase, creatine kinase and troponin 1 that were related to severity of disease [2] [7] [46]. These latter serum markers are indicative of liver, kidney and heart injury and are consistent with clinical findings on COVID-19 [2] [7] [44] [46].

Increased levels of C-reactive protein and erythrocyte sedimentation were also routinely found, suggesting endothelial cell damage in COVID-19 disease [44] [46] [47]. The COVID-19 patients requiring intensive care (ICU) also showed significant differences in prothrombin clotting time and increases in the presence of D-dimers (fibrin degradation fragments) [2]. There were differences also between surviving and non-surviving COVID-19 patients in ICUs [48]. Thrombotic complications have been a common finding in COVID-19 patients, with increases in acute pulmonary embolism, deep-vein thrombosis and systemic arterial embolism, despite intensive thromboprophylaxis [49]. It has been especially important to control blood hemodynamics in COVID-19 patients, especially in those patients requiring ventilation [47] [48] [49].

Similar to COVID-19 disease, patients with *mycoplasma* infections, especially *M. pneumoniae* infections, show significant increases in serum aspartate amino-

transferase, alanine aminotransferase as well as increases in lactate dehydrogenase [50]. As found in COVID-19 disease, this suggests that organ damage to liver and possibly other organs by the infectious process could be enhanced by the presence of mycoplasma or other bacteria. Also, high levels of C-reactive protein are typical in *M. pneumoniae* infections, and the ratios of C-reactive protein to procalcitonin were found to be predictive for mycoplasma-induced pneumonia [51]. Similar to COVID-19 patients, common findings in *M. pneumoniae* infections are thrombocytopenia, widespread platelet aggregation and hemolytic anemia [52].

6. Biotoxins and Host Responses

Various infective agents have evolved with different strategies to evade host non-immune and immune mechanisms that have been developed to inhibit infections. Simple RNA viruses use explosive replication to outpace host response mechanisms, but they have also evolved with particular strategies to deal with host responses, such as innate host responses and adaptive immune responses. SARS-CoV virus components or their replication intermediates are first recognized by host innate response systems using Pattern Recognition Receptors (PRRs) present in the cytosol and on various cellular membranes. These PRRs recognize viral Pathogen Associated Molecular Patterns (PAMPs) or viral structures with unique structural characteristics and initiate anti-infective responses [53] [54].

While SARS-CoV viruses attempt to evade host innate recognition and response systems, host cells that detect SARS-CoV viruses turn on production of cytokines, chemokines and interferon-stimulated gene (ISG)responses to counter SARS-CoV infections [54] [55]. This will be considered in the context of fatal infections in the next section. To counter innate immune signaling, SARS-CoV viruses encode several proteins that antagonize the host response to prevent activation of antiviral systems inside host cells and prevent host interferon responses [54].

Mycoplasmas use various virulence mechanisms to survive during their pathogenic development [56] [57]. Inside cells they compete for cellular nutrients and metabolites, and in this way they can depletehost precursor molecules and disrupthost metabolic and synthetic pathways [56]. They also secrete some of their own enzymes, such as lipases, proteases, nucleases and other enzymes, that can disrupt and interfere with host structures and metabolites [57] [58]. Mycoplasmas can also stimulate the generation of hydrogen peroxide and superoxide radicals that damage host cellular membranes, mitochondria and other structures [56].

Pathogenic mycoplasmas can synthesize degradative enzymes that can damage tissues and cause pathogenic changes, such as secondary necrosis [59]. They can also cause tissue damage with the morphological characteristics of apoptosis, such as chromatin condensation, as well as necrosis, with characteristic loss of

membrane integrity and organelle swelling [60]. Arginine deaminase is an example of a growth-inhibitory mycoplasma-produced enzyme that inhibits the growth of human T-cells. This enzyme can suppresses IL-2 production and receptor expression in T-cells stimulated by non-specific mitogens. It can also produce the morphologic features of dying cells, such as DNA fragmentation that is seen during apoptosis [42]. This enzyme has been followed in patients with community-acquired pneumonia as a possible marker for *M. pneumoniae* infections [61].

Some *Mycoplasma* species can directly cause host cell death, but a more common feature of pathogenic mycoplasma infections is the induction of host cytokines [62]. Indeed, cytokine-inducing activity is a general feature of most, if not all, pathogenic *Mycoplasma* species, and this important topic for COVID-19 disease will be discussed in the next section. The cell death effects of mycoplasmas are usually mediated by lipid-associated molecules (lipoproteins), and they are not associated with decreases in mitochondrial trans-membrane potential or inhibited by pre-incubation with the drug N-acetylcysteine, which is typically found in TNFα-mediated apoptosis. Rather, a non-lipid-associated protein (15 - 30 kDa) was found to cause mycoplasma-mediated cell death [63].

Similar to SARS-CoV viruses, pathogenic mycoplasmas cause cardiovascular and pulmonary manifestations that can result in extreme patient morbidity and death [57] [61] [62]. Several examples of cardiovascular and pulmonary tissue damage have been reported as due to vascular occlusion via thrombosis and the formation of vascular immune complexes. Pathogenic mycoplasma-caused vascular occlusion has been reported for heart, lung, kidney, brain and other organs [60] [62].

Pathogenic mycoplasmas also release biotoxins that directly damage cells and tissues and stimulate host innate response systems [58] [62] [63] [64]. A *Mycoplasma pneumoniae*-released biotoxin, called the community-acquired respiratory distress syndrome toxin (CARDS), has been isolated and found to bean ADP- and protein-ribosylating as well as a vacuole-causing cytotoxin [65]. The effects of this biotoxin also include alterations of enzymes and other proteins of various metabolic pathways. As mentioned above, this biotoxin activates innate immunity, and this is mediated through the NLRP3 inflammasome complex, which ultimately causes cell-release of IL-1. It also stimulates hyper-inflammation and tissue damage, among other pathologic effects, and it appears to be responsible, in part, for pulmonary inflammation along with cytokine release. Clinically it causes significant airway dysfunction, which is usually seen as loss of ciliary function of the respiratory epithelium and includes lung cell vacuolization, lung cell rounding/distortion and disruption of pulmonary epithelial integrity. It may be responsible for respiratory failure and some of the fatal outcomes that have been found in acute *M. pneumoniae* infections [66].

7. Cytokines and Cytokine Storms

Excess inflammatory cytokine and chemokine production and release into the

surrounding tissue and the circulatory system ("cytokine storm") can be seen during severe infectious disease progression, and it is often found in fatal cases of viral infections. It is caused by a severe and excessive immune response initiated by a positive feedback cycle between various cytokines and immune cells.

COVID-19 disease progression to severe hypoxia, pulmonary edema, accumulation of inflammatory cells in the lungs, ARDS and eventually organ failure often ends in a lethal outcome with high mortality rates [2] [3] [5] [6] [7]. Consistent with this lethal progression is the increasing induction and production of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, IL-7, IL-8, IL-10, tumor necrosis factor-alpha (TNFα) and other cytokines and chemokines, all of which have been found in SARS-CoV viral infections [54] [55]. Compared to healthy, non-symptomatic adults the levels of plasma cytokines (IL-1β, IL-RA, IL-7, IL-8, IL-9, IL-10, TNFα and other cytokines and chemokines) were higher in both non-ICU and ICU patients with COVID-19 disease compared to controls [2]. Importantly, recent studies have shown significantly higher levels of inflammatory cytokines (IL-2R, IL-6, IL-8, IL-10, TNFα) in ICU non-survivors compared to ICU survivors of COVID-19 disease [67]. This suggests that excessive, multiple cytokines produced during progressive disease or 'cytokine storm' contributes to the fatal outcome seen in many COVID-19 patients.

Cytokine/chemokine release at tissue sites, such as the inflammatory cytokines and chemokines found at exaggerated levels in lung tissue during RNA virus infections, is particularly difficult to deal with in animals and in humans [68] [69]. There are over 150 different pro-inflammatory and anti-inflammatory cytokines, chemokines, interferons, growth factors and other tissue factors that are synthesized and released into tissues and the blood during a vigorous immune system response, and these signaling molecules can cause high fever, redness, swelling, fatigue, nausea and other symptoms [68] [69] [70].

At the cellular and tissue levels SARS-CoV viruses and the virus-induced cytokine storms that are caused by these viruses result in significant damage to tissues, especially lung tissue. This pulmonary damage can be observed as diffuse injury to alveolar epithelial cells, fibroblasts and alveolar macrophages. Specifically, the damage has been characterized as hyaline membrane formation, desquamation of pneumocytes, edema and inflammatory cell infiltration, among other adverse effects [71].

During the infection process lung cells secrete various cytokines and chemokines that induce fibroblast activation, extracellular matrix deposition and alveolar epithelial damage. Such aberrant response, along with excessive cytokine production, are not unique to RNA viruses; they have been associated with the pathogenesis of a variety of non-infectious and infectious diseases, from viral infections to neurodegenerative disorders [70].

Mycoplasma infections also result in the production of inflammatory cytokines, including IL-1β, IL-2, IL-6, IL-8 and TNFα as well as various interferons and leukocyte growth factors [57] [62] [63] [64]. In fatal cases of *M. pneumoniae*

cytokines, such as IL-18 but not interferon, were found to be significantly higher in, for example, patients with fulminant pneumonia [12]. During infection by *M. pneumonia* the production of cytokines (IL-1β, IL-6, IL-10, TNFα, among others) increases markedly, and this is thought to be an indication of tissue damage [72]. In the case of *M. pneumoniae* such damage has been directly related to the release of various inflammatory cytokines, chemokines and other inflammatory molecules and the subsequent tissue and immune responses to these molecules [72].

8. Antibiotics, Anti-Malarial and Other Treatments

In describing some of the treatments used to fight COVID-19 disease, we will only discuss here those commonly used treatments that relate to possible bacterial co-infections or activated latent bacterial infections. Although the consequences of SARS-CoV-2 coronavirus anti-viral and other treatments and general ICU supportive procedures are fundamentally important in caring for COVID-19 patients, that is not the purpose of our contribution. The use of anti-viral and other drugs and ICU supportive procedures have been extensively reviewed by others, and this discussion will not be repeated here. Thus the reader is referred to other recent articles for information on primary and ICU supportive treatments useful in the management of COVID-19 disease [2] [6] [7] [28] [29] [44] [47] [48].

8.1. Antibiotics and COVID-19

Since mycoplasmas do not have cell walls, the antibiotics that act on cell wall synthesis, such as β-lactams (penicillins, cephalosporins, among others),are ineffective against mycoplasmas [24] [62] [64] [73] [74]. Thus mycoplasmas are often treated with anti-microbials that act on their metabolism, replication, synthetic machinery or other specific bacterial targets, even though the actions of these drugs are mainly bacterostatic [24] [62] [74]. Since most mycoplasmas are sensitive to tetracyclines (doxycycline, minocycline, among others) or macrolides (azithromycin, clarithromycin, among others), with some notable exceptions [62] [75] [76] [77], these are often used for frontline treatment of *Mycoplasma* species, and quinolones (ciprofloxacin, sparfloxacin, levofloxacin, among others), are often used as alternative treatments [62] [76].

In general viruses are not susceptible to antibiotics, but particular antibiotics have been used during viral infections to treat bacterial co-infections or latent infections. This is common in cases of adult community-acquired pneumonia where *Mycoplasma* species were often the most frequent type of bacterial infection found in cases identified as viral pneumonia [78].

In addition, some macrolide antibiotics, such as azithromycin, have anti-viral activities against specific viruses, such as rhinoviruses identified in virus-associated pulmonary conditions found in cystic fibrosis [79] and zika virus recovered from developing human brain tissue [80]. In bronchial epithelial cells pre-

treatment with azithromycin reduced rhinovirus replication as well as asthma exacerbations and other complications, as estimated by the synthesis of pro-inflammatory cytokines, interferon-β responses and increases in rhinovirus-induced pattern recognition receptor [79].

Other macrolides may be useful in the treatment of respiratory viral infections due to their effects on pulmonary cells. In this situation the positive effects of macrolides have been attributed, in part, to their anti-inflammatory and immunomodulatory effects [81] [82] [83]. Although macrolides have been shown to be efficacious in treating some infections, their use comes with some possible risk of cardiac complications, such as QT prolongation [84].

In COVID-19 disease antibiotics have been used mainly as a part of supportive care and prevention of super-infection, without identification of possible bacterial co-infections or activation of latent bacterial infections [2] [7] [44] [47] [48]. In some treatment studies on COVID-19 disease an antibiotic (azithromycin) was used with an anti-malarial drug (hydroxychloroquine). For example, this combination was used in Marseille, France in an open label non-randomized clinical trial, but the azithromycin was added mainly as a part of supportive care [85]. The results of this preliminary study will be discussed in the next section.

Another antibiotic active against mycoplasmas, doxycycline [62] [64] [73], might also be useful in COVID-19 because of its potential binding to rRNA and inhibition of microbial protein synthesis [86]. Indeed, doxycycline has proved to be an important option for chronic mycoplasma infections resistant to other treatments [62] [73], and its effectiveness in COVID-19 care might be more related to its suppression of bacterial growth than any anti-viral action.

8.2. Anti-Malarial Drugs and COVID-19

The interesting use of the anti-malarial drug, hydroxychloroquine, in the treatment of COVID-19 disease was reported by Gautret *et al.* [85]. This followed from an earlier study on the suppressive effects of chloroquine on SARS-CoV infection of Vero E6 cells in culture [87]. Chloroquine and hydroxychloroquine have many uses because of their anti-inflammatory and potential chemo-sensitization properties; they have been used widely to treat various human diseases, such as malaria and amoebiosis, without significant adverse effects [88]. The main anti-parasitic and anti-viral effects of the chloroquines are thought to occur by the alkylization of cellular endosomes, Golgi and lysosomes, and possibly also by affecting phospholipid metabolism and zinc ion levels to modify parasite and virus entry [89]. In addition, chloroquines block BK channels that are essential in proinflammatory responses that can lead to cytokine storms [90].

In China chloroquine phosphate has been used to treat COVID-19 pneumonia patients. Although a recent preliminary report on this lacks detail and analysis, it was stated that chloroquine could be a breakthrough in COVID-19 treatment [91]. In this study, patients receiving chloroquine phosphate showed less exacerbation of pneumonia, and they had improved lung imaging compared to

control treatment. In addition, severe adverse reactions to chloroquine phosphate were not found in these patients [91]. In the French study using hydroxychloroquine and azithromycin, viral carriage was reduced significantly over a 6-day study with hydroxychloroquine, and the addition of azithromycin was significantly better than hydroxychloroquine alone [85]. In a recent randomized clinical trial using low (450 mg) and high (600 mg) dose chloroquine as adjunct therapy for COVID-19 patients viral RNA was detected at about the same prevalence in both groups: 31/41 (low dose) and 31/40 (high dose). However, by day 13 fatalities in the high-dose group were higher (39%) than in the low-dose group (15%), most likely because of a higher incidence of heart problems in the high-dose group [92]. Thus use of chloroquine or hydroxychloroquine for COVID-19 disease may come at a cost—a higher incidence of coronary problems [92]. Even with these limitations, hydroxychloroquine and chloroquine phosphate have been proposed to be potentially useful experimental drugs for the treatment of some COVID-19 patients [93].

8.3. Other Treatments in COVID-19

In addition to anti-viral drugs that target SARS-CoV-2 viral replication, other treatment approaches for COVID-19 disease include methods to inhibit viral attachment, fusion and entry into cells, suppression of inflammatory responses, vaccines and convalescent plasma treatments [94] as well as combinations of conventional and alternative medicine [37] [38] [94]. For example, various combinations of anti-viral, anti-inflammatory and other drugs along with anti-oxidants, zinc ion, and other approaches, such as the use of molecular hydrogen to help control inflammation and oxidative damage, have been proposed [95]. For the most part, the current approaches used to develop new treatments for COVID-19 disease do not take into account the possibility of bacterial co-infections or activation of latent bacterial infections.

9. Final Comments

Our hypothesis has been that infections like *Mycoplasma* and other bacterial species (*Chlamydia pneumoniae*, among others) could be contributing to the morbidity and mortality seen in COVID-19 disease. Infections like *M. pneumoniae*, *M. fermentans* and other *Mycoplasma* species are known to cause lethal diseases on their own in some patients, so when present with SARS-CoV-2 infections, they could be significantly contributing to COVID-19 mortality. In other diseases caused by RNA viruses, such as HIV-1, *M. fermentans* and *M. penetrans* co-infections have been proposed to be important co-factors in the development of fatal disease [96] [97]. This could also be, in part, the reason that some patients with COVID-19 progress to a fatal disease.

Mycoplasma and other similar bacterial infections should be carefully analyzed in critical COVID-19 patients. If such tests are positive, these patients should be treated accordingly [62] [64] [73]. In order to determine the possible

role of *Mycoplasma* species in the progression of COVID-19 disease to a fatal disease course, patients who are positive for such infections should be compared to patients that do not have these infections at various stages of the disease process to see if the SARS-CoV-2 virus can activate latent *Mycoplasma* species or enhance sub-clinical mycoplasma infections and promote COVID-19 disease morbidity and progression to a fatal outcome.

Note Added in Proof

Since we prepared this manuscript, there have been recent contributions, mostly brief preprint reports or letters that support our hypothesis. Charkraborty and Das [98] discussed the possibility that anaerobic bacteria, including *Mycoplasma* species, could be causing secondary infections in COVID-19 disease. They have proposed that such infections may be altering hemoglobin degradation and producing metabolites that affect hypoxia in progressing COVID-19 patients [98]. Stricker and Fesler [99] suggested that patients who have COVID-19 disease should not progress to a fatal outcome, if their therapy includes combinations of antibiotics (including minocycline or doxycycline) used for tick-borne infections [99]. As we discussed previously, Lyme disease patients often have mycoplasma co-infections that are sensitive to minocycline and doxycycline [62] [75].

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Disclosures

One of us (G.L.N.) is a part-time consultant to Nutritional Therapeutics, Inc., Naturally plus USA and UNVIA Naturally plus Taiwan.

Conflicts of Interest

The authors have no conflicts of interest to declare regarding this contribution.

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Clinical Observations on the Effects of a Dietary Supplement (GI Regenerate™) on Patients' Gastrointestinal Symptoms and Quality of Life Assessments

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Abstract

Background: Different treatments have been developed and used to control symptoms and improve quality of life in patients with digestive diseases and disorders. Although the use of drugs or alternative approaches has improved symptom severity in some but not all patients, often these improvements were not sustainable. **Objectives:** An open label clinical study was initiated to determine if oral capsules containing a dietary supplement of herbs and oils (GI Regenerate™) could reduce self-reported gastrointestinal symptoms and improve quality of life (QOL) indicators in patients with gastrointestinal conditions. **Methods:** Participants included 50 patients (40 females and 10 males) of mean age of 51.1 ± 12.7 years (range, 24 - 77 years) with a diagnosis of a gastrointestinal disorder or gastrointestinal symptoms. These patients consumed five soft-gels containing the test supplement 30 minutes before each meal for 90 days. Symptoms were evaluated by medical staff, and patient health status was self-reported using a validated quality of life questionnaire (Quality of Life Digestive Survey) designed for functional digestive disorders. Exit interviews (Patient Global Impression of Change, PGIC) were conducted by the medical staff. **Results:** Participants in the study responded with improved symptom severities and QOL scores to the test dietary supplement within the 90 day period; most improvements occurred within 20 days on the test dietary supplement. By the end of the study there were significant overall global improvements in the symptoms and QOL health surveys ($p = 0.0183$),

with significant improvements in symptom discomfort (p = 0.0004), daily activities (p = 0.029) and anxiety (p = 0.018). In contrast, there were insignificant improvements in diet (p = 0.398), sleep (p = 0.136), health perception (p = 0.686), coping with the disease (p = 0.309) and impact of stress (p = 0.785). Using the PGIC exit interview that measured each patient's impression of overall global change in symptoms and QOL these data also indicated overall significant improvements in symptoms and in satisfaction with the test supplement (moderately better improvements in symptoms and QOL or score of 4.8 ± 0.169, p < 0.0001). There were no significant differences in the responses between males and females, and no significant differences between older (>50 years) versus younger (<50 years) subjects. There were also no safety issues that arose during the trial. **Conclusions:** The GI Regenerate™-natural dietary supplement safely and significantly reduced gastrointestinal symptoms and improved quality of life in subjects with a broad spectrum of gastrointestinal disorders and symptoms.

Keywords

Gastrointestinal Symptoms, Quality of Life, Dietary Supplement, Digestive Disorders, Herbal Remedies, Dietary Oils

1. Introduction

There is a rather large burden to the United States population of morbidity, mortality and cost due to gastrointestinal (GI), liver and pancreatic diseases and disorders [1] [2], and this appears to be true in other nations as well [3]. With its aging population, the United States faces an increasing prevalence of digestive diseases over time [2]. This is likely to result in an overall worsening of the productivity and quality of life (QOL) in the aging population [4] [5].

Different treatments have been developed and used to control symptoms and improve QOL in digestive diseases and disorders [6] [7]. Among the pharmaceutical treatments that are commonly used, such as corticosteroids, aminosalicylates, antibiotics and immunosuppressive drugs, improvements in symptoms have been found, but not in every patient, and often these improvements are not sustainable. Also, the drugs that are often prescribed can have adverse effects in some patients. Thus complementary or alternative medicine approaches have been used to avoid the adverse effects of drug treatments and improve treatment outcomes [6] [7].

Among the alternative medical approaches to the treatment of GI diseases and disorders is the use of herbal combinations, and this has proved beneficial for many patients [8] [9] [10]. There is a rich history that goes back thousands of years of using single and multiple herbal formulations to treat digestive diseases and disorders [8] [10]. In the United States, a large proportion of patients with digestive disorders have tried some form of herbal treatment [10] [11]. The most commonly used herbal treatments for digestive diseases and disorders in the US

have their origin in traditional Chinese medicine [10].

One such combination of herbs and oils that has been used for years in China to treat digestive disorders has been utilized in the current study. This same combination dietary supplement has had different names (GIC, MEBO Gastrointestinal Capsule, Dr. Xu's GI Formula, or more recently GI Regenerate™), and it has been the subject of several scientific and clinical studies in China. These studies include: survival and growth promotion of intestinal and stomach epithelial cells [12] [13], clinical treatment studies on ulcerative colitis [14], gastroesophageal reflux disease [15], gastric ulcers [16], peptic ulcers [17], and repair of gastrointestinal damage due to ethanol [18] or infection [19].

Using a validated QOL questionnaire for functional digestive disorders [20] and patient global impression of change scores (PGIC) taken during exit interviews by contributing physicians this same dietary supplement formulation, or GI Regenerate™, has been examined for its use in treating digestive disorders and symptoms in North American patients.

2. Materials and Methods

2.1. Materials

GI Regenerate™ is a patented natural supplement containing a mixture of herbal ingredients and edible oils. It contains stigmasterol, campesterol, beta-sitosterol, chalinosterol, clionasterol, brassicasterol, alpha-spinasterol, daucosterol, desmosterol, poriferasterol and an edible wax [21]. This base mixture was placed (250 mg each) into soft gel capsules. The natural dietary supplement used in the clinical study was provided by MEBO Life Sciences, Brea, California.

2.2. Methods

An open label, independent Institutional Review Board (IRB)-approved study was initiated using subjects recruited from Southern California with formally diagnosed digestive disorders and diseases, such as ulcerative colitis, gastritis, esophagitis, gastroesophageal reflux disease (GERD), Crohn's disease, irritable bowel syndrome (IBS), or other digestive disorders. The study recruitment was limited to patients attending the Center for New Medicine, Irvine, California who volunteered for the study. The number of subjects was determined by the number of patients who volunteered and could be adequately scheduled, examined and treated by available staff during the trial period of January 2019 to January 2020.

The 40 females and 10 males recruited to the study presented with a variety of signs and symptoms related to digestive disorders (**Table 1**). Exclusionary criteria included subjects who were taking immunosuppressive drugs, or had cognitive impairment, or were pregnant, lactating or below the age of 18 years. Each subject was directed to take 5 capsules of GI Regenerate™ 30 min before meals 3X per day for the 90-day study period. Participants were advised not to change any of their daily medications, diet or routine during the study.

Table 1. Diagnoses/symptoms of subjects in the clinical study.

Diagnosis/Symptom*	*N*
Female Subjects (*Total*)	40
Abdominal bloating	26
Abdominal pain	22
Celiac disease	2
Constipation	25
Crohn's disease	3
Diarrhea	23
Fatigue	14
Flatulence	9
Food allergy	7
Gastric pain	3
Gastritis	10
GERD	17
IBS	16
Intestinal malabsorption	3
Nausea	10
Obesity	1
Regurgitation	4
Ulcerative colitis	7
Male Subjects (*Total*)	10
Abdominal Bloating	2
Abdominal pain	6
Celiac disease	-
Constipation	7
Crohn's disease	1
Diarrhea	9
Esophagitis	1
Fatigue	3
Flatulence	2
Food Allergy	3
Gastric pain	6
Gastritis	-
GERD	6
IBS	1
Intestinal malabsorption	-
Nausea	1
Obesity	1
Regurgitation	1
Ulcerative colitis	1

*Subjects may have more than one diagnosis and have multiple symptoms. IBS, irritable bowel syndrome; GERD, gastroesophageal reflux disease.

Study subjects were monitored at various times using a validated patient questionnaire for functional digestive disorders and QOL (Appendix Figure 1 in reference [20]). The data were normalized to baseline and analyzed as: a) Overall Global Scores; and subsets of data were normalized to baseline and analyzed as: b) Daily Activities Scores, c) Symptom Discomfort Scores, d) Anxiety Scores, e) Diet Scores, f) Sleep Scores, g) Coping with Disease Scores, h) Health Perception Scores, and i) Stress Impact Scores [20].

Subjects were also subjected to exit examination and surveys conducted by professional staff physicians of the Center for New Medicine of Irvine, California. In this (PGIC) analysis participants were asked whether their overall changes in symptom severity and QOL were very much better (score of 6), moderately better (score of 5), a little better (score of 4), no change (score of 3), a little worse (score of 2), moderately worse (score of 1) or very much worse (score of 0) (**Appendix Figure A1** of this paper). The mean satisfaction scores were determined and analyzed statistically.

2.3. Statistical Analysis

For statistical analysis we used generalized estimating equations (GEE) for the regression parameters as introduced by Liang and Zeger as a method for estimation of regression model parameters when dealing with correlated data [22] [23]. Generalized estimating equations (GEE) are a convenient and general approach to the analysis of several kinds of correlated data. The main advantage of GEE resides in the unbiased estimation of population-averaged regression coefficients despite possible misspecification of the correlation structure. Our longitudinal research was aimed at describing the marginal expectations of the outcome as a function of the predictors [24].

The objective of analyses that we have done and performed were to examine: (1) whether the QOL scores differed over the study time points; (2) whether the QOL scores differed over the study time points between males and females; and (3) whether the QOL scores differed over the study time points between age < 50 and age ≥ 50. Data were analyzed with significance defined as $p < 0.05$ and presented as mean data with 95% confidence levels.

The exit survey (PGIC) was conducted with 28 subjects, and satisfaction scores were calculated and analyzed by a one-sided, one sample t-test. In this analysis a significant overall improvement in exit scores would be a composite satisfaction score greater than 3.0. All of the statistical analyses were performed independently by the Statistical Unit of the Division of General Medicine, Department of Medicine, University of California, Irvine.

2.4. Safety Issues

The safety of patients was carefully monitored during the trial. Any issues of adverse reactions to the test supplement were carefully recorded and monitored during the trial. Potential changes in blood chemistry were monitored each

month during the clinical study using the NutrEval™ diagnostic blood evaluation panel (Genova Diagnostics, Asheville, NC). In this panel standard blood chemistry and a panel of blood levels of antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, and amino acids were monitored at the beginning and each subsequent month during the trial period.

3. Results

3.1. Participants in the Study

There were 50 participants in the IRB-approved clinical study (40 females, 10 males). They had a mean age of 51.1 ± 12.7 years (range, 24 - 77 years) and presented with a diagnosis of a gastrointestinal disorder. A summary of the participants and their presentation with a variety of digestive disorders and diseases (with multiple gastrointestinal symptoms) is summarized in **Table 1**.

3.2. Quality of Life Determinations

Using the validated digestive disorders questionnaire of Chassany, *et al.* [20] patients were examined for their responses in each survey category every 10 days during the 90-day test period (**Figure 1**). After the 90-day period, the analyzed results of the study indicated that there were significant overall global improvements in the health surveys ($p = 0.0183$) (**Figure 1(a)**), with significant improvements in symptom discomfort ($p = 0.0004$) (**Figure 1(b)**), daily activities ($p = 0.029$) (**Figure 1(c)**) and anxiety ($p = 0.018$) (**Figure 1(d)**). In contrast, there were insignificant improvements in diet ($p = 0.398$) (**Figure 1(e)**), sleep ($p = 0.136$) (**Figure 1(f)**), health perception ($p = 0.686$) (**Figure 1(g)**), coping with the disease ($p = 0.309$) (**Figure 1(h)**) and impact of stress ($p = 0.785$) (**Figure 1(i)**). Most health response improvements over baseline occurred within 20 days from initiating the dietary supplement (**Figures 1(a)-(d)**).

Based on the results from the GEE models, regression parameters indicated that the improvements in overall global symptoms and QOL scores were consistent and occurred with a low degree of variance. The estimated changes from baseline of the eight dimension scores are shown in **Table 2**. The dimension scores included: daily activities (DA), anxiety (AN), diet (DI), sleep (SL), discomfort (DT), health perceptions (HP), coping with disease (CD), and impact of stress (IS). In addition, two overall measures, the estimated change from baseline in the global score (GS) and an alternative scoring of the global score (Alt GS) are also displayed. The Table illustrates the low degree of variance in estimated changes from baseline in dimension scores and global scores over the 10 survey time points.

3.3. Exit Interviews

Exit interviews (PGIC) with each participant were conducted by the clinical study physicians (**Appendix Figure A1**). The exit interviews indicated that the

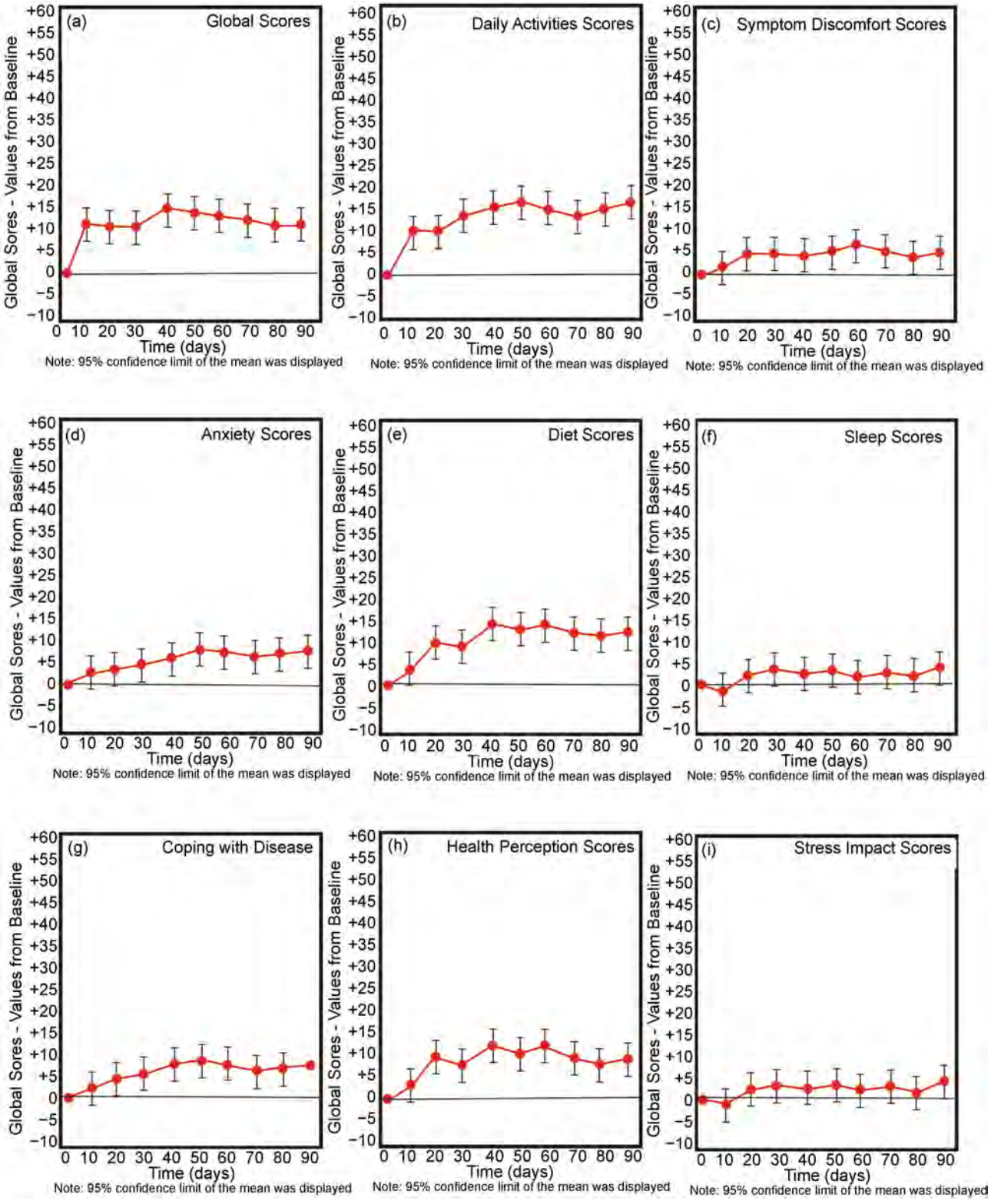

Figure 1. Digestive disorders questionnaire results. Combined results of the study (Global Scores) and subparts of the study over a 90-day period are presented. Results indicate normalized scores (mean scores minus baseline scores; brackets indicate 95% confidence levels of the means). Improvements in normalized scores are indicated by increases in normalized score values presented in the figure. Panels indicate Combined Global Scores (a), Daily Activities Scores (b), Symptom Discomfort Scores (c), Anxiety Scores (d), Diet Scores (e), Sleep Scores (f), Coping with Disease Scores (g), Health Perception Scores (h), Stress Impact Scores (i).

Table 2. The estimated changes from baseline of the dimension score at each survey time from the GEE models.

The Estimated Changes from Baseline of the Dimension Scores at Each Study–Time during Survey										
Survey time point (days)	DA Estimates (95%CI)	AN Estimates (95%CI)	DI Estimates (95%CI)	SL Estimates (95%CI)	DT Estimates (95%CI)	HP Estimates (95%CI)	CD Estimates (95%CI)	IS Estimates (95%CI)	GS Estimates (95%CI)	Alt GS Estimates (95%CI)
0	0.02 (−0.28, 0.31)	0.02 (−0.2, 0.24)	0.11 (−0.18, 0.4)	−0.06 (−0.28, 0.16)	−0.05 (−0.2, 0.11)	−0.02 (−0.13, 0.08)	−0.05 (−0.27, 0.17)	−0.01 (−0.13, 0.12)	−0.02 (−0.17, 0.12)	−0.02 (−0.17, 0.12)
10	12.53 (6.57, 18.5)	9.79 (4.98, 14.61)	2.6 (−1.54, 6.75)	1.85 (−1.36, 5.06)	3.7 (−0.52, 7.92)	−1 (−4.54, 2.54)	3.62 (−4.06, 11.3)	−1.56 (−6.86, 3.73)	4.71 (2.1, 7.31)	4.66 (2.2, 7.11)
20	11.22 (5.53, 16.91)	9.8 (4.36, 15.24)	5.11 (−0.08, 10.3)	4.68 (0.31, 9.05)	10.65 (5.79, 15.51)	1.46 (−3.25, 6.18)	9.69 (2.91, 16.47)	−1.53 (−6, 2.94)	7.49 (4.15, 10.83)	7.19 (3.96, 10.42)
30	11.27 (4.75, 17.79)	12.19 (5.91, 18.47)	5.54 (0.01, 11.08)	5.42 (0.22, 10.61)	10.12 (5, 15.24)	3.11 (−0.95, 7.17)	5.31 (−1.45, 12.06)	−1.09 (−7.1, 4.91)	7.53 (3.89, 11.18)	7.4 (3.91, 10.89)
40	14.55 (8.52, 20.57)	14.64 (8.99, 20.28)	5.03 (−0.92, 10.97)	7.25 (1.87, 12.64)	15.85 (11.05, 20.66)	2.89 (−1.45, 7.22)	8.54 (1.72, 15.36)	−1.74 (−7.59, 4.11)	9.8 (5.9, 13.7)	9.74 (6.05, 13.43)
50	13.71 (7.13, 20.28)	16.91 (10.22, 23.61)	6.94 (0.69, 13.2)	9.81 (4.62, 15.01)	14.67 (10.08, 19.27)	3.53 (−1.04, 8.1)	10.98 (2.58, 19.38)	2.74 (−3.61, 9.09)	10.94 (6.88, 15)	10.65 (6.78, 14.51)
60	13.14 (6.75, 19.54)	16.03 (9.16, 22.9)	8.76 (2.54, 14.98)	9.68 (3.99, 15.36)	17.12 (11.48, 22.75)	1.42 (−2.98, 5.82)	8.78 (0.7, 16.87)	2.3 (−3.4, 8)	10.73 (6.75, 14.7)	10.74 (6.84, 14.63)
70	12.64 (6.81, 18.48)	14.51 (6.65, 22.37)	7.12 (−0.04, 14.28)	8.12 (2.29, 13.96)	14.16 (8.34, 19.99)	2.09 (−2.55, 6.72)	10.48 (1.7, 19.26)	1.27 (−5.69, 8.24)	9.89 (5.11, 14.68)	9.66 (5.11, 14.2)
80	10.93 (4.52, 17.34)	16.35 (7.66, 25.04)	6.31 (0.37, 12.24)	8.98 (3.78, 14.17)	13.11 (7.09, 19.13)	0.9 (−3.64, 5.44)	9.55 (2.11, 17)	0.74 (−7.84, 9.31)	9.44 (5.25, 13.64)	9.02 (4.87, 13.16)
90	10.88 (3.16, 18.6)	17.25 (8.84, 25.66)	7.13 (0.7, 13.55)	10.15 (4.11, 16.18)	13.83 (7.31, 20.35)	3.9 (−1.07, 8.87)	8.08 (−1.73, 17.88)	2.6 (−4.73, 9.94)	10.19 (5.66, 14.72)	9.83 (5.42, 14.24)

Abbreviations: DA, daily acitivites; AN, anxiety scores; DI, diet scores; SL, sleep scores; DT, symptom discomfort scores; HP, health perception scores; CD, coping with disease scores; IS, impact scores; GS, global scores; Alt GS, alternate global scores; CI, confidence intervals.

patients' impression of overall global change in symptoms and QOL showed significant improvements in satisfaction with the test supplement (moderately better improvements in symptoms and QOL, or a score of 4.8 ± 0.169, $p < 0.0001$).

3.4. Safety of the Study

There were no safety issues that came up during the clinical trial. In support of this the NutrEval™ diagnostic blood evaluation panels showed no significant changes in blood chemistry and levels of blood antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, and amino acids during the study.

4. Discussion

The dietary test supplement used in the present clinical study (now called GI Regenerate™) has been used for years in China and other countries to treat patients with a variety of gastrointestinal disorders and diseases [14]-[19]. These clinical studies were dependent on this dietary supplement repairing gastrointestinal damage. To demonstrate the effects of the test dietary supplement on stimulating gastrointestinal epithelial cell survival, growth and regeneration, some

experimental studies were initiated. After excision and *in vitro* culture of organ explants of murine stomach and intestinal tissues in medium containing fetal bovine serum, addition of the test dietary supplement was shown to stimulate epithelial cell survival, growth and differentiation, whereas the cells in explant cultures without the dietary test supplement began to die and never formed viable cell colonies [12] [13].

Consistent with the findings in China on the clinical benefits of using the oral test dietary supplement to treat ulcerative colitis [14], gastroesophageal reflux disease [15], gastric ulcers [16], peptic ulcers [17], GERD [23] and gastrointestinal damage due to ethanol [18] or infection [19] we found that North American patients with a variety of gastrointestinal disorders and symptoms (**Table 1**) responded positively to the test dietary supplement GI Regenerate™. These positive responses were collected using the validated digestive disorders questionnaire of Chassany, *et al*. [20] over a 90-day period. The results indicated that male and female patients with IBS, GERD, Crohn's disease, celiac disease, ulcerative colitis, gastritis, and digestive symptoms, such as abdominal bloating and pain, gastric pain, constipation, diarrhea, fatigue, flatulence, nausea, regurgitation and food allergies and malabsorption, improved significantly during the test period ($p = 0.0183$), with significant QOL improvements in symptom discomfort ($p = 0.0004$), daily activities ($p = 0.029$) and anxiety ($p = 0.018$).

Our results using the validated digestive disorders questionnaire were confirmed in the PGIC exit surveys where patients indicated moderately better improvements in symptoms and QOL ($p < 0.0001$) at the end of the study. Thus we have confirmed the benefits of taking oral capsules of GI Regenerate™ found in previous studies on the improvements in gastrointestinal symptoms in patients with digestive disorders and diseases [14]-[19].

There were no safety concerns that came up during the trial. Patients did not report issues with the GI Regenerate™ oral supplement, and blood chemistry analyses every month during the trial on every subject using the NutrEval™ diagnostic blood evaluation panel did not indicate any abnormalities in levels of blood antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, or amino acids during the study. Thus we concluded that the GI Regenerate™ oral supplement was safe and effective for use in treating gastrointestinal symptoms in early adults to the elderly.

Although the results of our clinical study were positive and generally significant statistically, there were obvious limitations of the trial. First, we note that although the numbers of females in our study were sufficient, we had less access to male patients. Thus the numbers of males in our study (10) were much lower than the numbers of females (40). Future studies should contain more balanced numbers of males and females. Also, the study was a preliminary open label study, not a robust, randomized, controlled clinical trial. There are few evidence-based clinical studies using randomized clinical trials on the use of Chinese dietary herbal supplements to treat digestive disorders [25] [26]. The results

presented here should stimulate the organization of a randomized, controlled clinical trial using GI Regenerate™ to test for improvements in symptoms in patients with digestive disorders and diseases.

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Disclosures

Garth L. Nicolson and Robert Settineri are part-time research consultants to Allergy Research Group, Inc., Naturally Plus USA, Inc. and Nutritional Therapeutics, Inc. There are no other disclosures.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Patient Name:
Subject Number:
Email:
Phone number:
Exit date:

Please choose the response below that best describes the overall change in your symptoms and quality of life since you started taking the study supplement.

- ❑ Very Much Better
- ❑ Moderately Better
- ❑ A Little Better
- ❑ No change
- ❑ A Little Worse
- ❑ Moderately Worse
- ❑ Very much Worse

Patient Signature: _____

Date: _____

Figure A1. Patient's Global Impression of Change (PGIC).

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Parametrization of Survival Measures, Part I: Consequences of Self-Organizing

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Abstract

Lifetime analyses frequently apply a parametric functional description from measured data of the Kaplan-Meier non-parametric estimate (KM) of the survival probability. The cumulative Weibull distribution function (WF) is the primary choice to parametrize the KM. but some others (e.g. Gompertz, logistic functions) are also widely applied. We show that the cumulative two-parametric Weibull function meets all requirements. The Weibull function is the consequence of the general self-organizing behavior of the survival, and consequently shows self-similar death-rate as a function of the time. The ontogenic universality as well as the universality of tumor-growth fits to WF. WF parametrization needs two independent parameters, which could be obtained from the median and mean values of KM estimate, which makes an easy parametric approximation of the KM plot. The entropy of the distribution and the other entropy descriptions are supporting the parametrization validity well. The goal is to find the most appropriate mining of the inherent information in KM-plots. The two-parameter WF fits to the non-parametric KM survival curve in a real study of 1180 cancer patients offering satisfactory description of the clinical results. Two of the 3 characteristic parameters of the KM plot (namely the points of median, mean or inflection) are enough to reconstruct the parametric fit, which gives support of the comparison of survival curves of different patient's groups.

Keywords

Self-Organizing, Self-Similarity, Avrami-Function, Weibull-Distribution, Survival-Time, Allometry, Entropy, Bioscaling

1. Introduction

The driving force of the overall spontaneous progressions in nature is the at-

tempt to minimize the actual energy and maximize the entropy in the actual processes. In this sense, life follows the basic thermodynamic laws: the living process continuously "burns" the incoming "nutrition". Only the energy-pump of the incoming sun-energy makes the difference: creates original gradients which are later divided into other inhomogeneities by spontaneous processes.

Life process tries to diminish the working energy of the sunlight by increasing the overall entropy of the environment. Living process lowers the electron energy by the oxidation producing outgoing (waste) final "products". The gradual loss of electron energy of the "nutrition" molecules is the energy to sustain life. Simply speaking, the living process is a dissipative entropy producer. As the Nobel laureate physiologist A. Szentgyorgyi states "Life is nothing but an electron looking for a place to rest" [1].

Living objects are open systems among various environmental surroundings, adapting themselves to the conditions around, forming self-organized structures [2], and forcing evolution [3]. The approach of complexity becomes a useful tool for the description of nature [4] [5]. Self-organization appears in various scientific problems [6]. The self-organization explains multiple structural and dynamical challenges in biology [7]; it is observed in broad range of research from the gene-regulatory networks [8], through the cells [9], to the general evolution of living objects [10].

The invariance of magnification (scale invariance, when the up or down magnification shows similar structures) is the form of self-similarity, which is a typical consequence of the self-organizing processes, [11] [12]. It has developed a new discipline, the fractal physiology [13] [14] [15]; where stochastic processes are applied instead of the deterministic actions, so the predictions of the distant future always have random, unpredictable elements.

Random stationary, stochastic, self-organizing processes form dynamic behaviors [16], define a spatiotemporal-fractal structure, which is self-similar both in space and time [17]. The spatiotemporal fractal structure is a fingerprint of the self-organizing [18], and especially characteristic for the living matter [19]. The basal metabolism as the energy consumption of the living objects has a central role in system biology [20] and describes the biosystems by definite properties [21]. According to the system biology:

- life is complexly organized in a wide range of magnification and different levels of interactions,
- life is self-regulated with various feedback processes,
- the living systems are open, dissipative objects with multilevel interactions with the environment,
- the activity of life processes has intensive cross-talks of different levels of its organization,
- the specific forms and properties are complexly environment dependent

These points are important for the universality of life, for the dynamic fluctuations and scaling too [22], and as a character of life, it could be used in its di-

agnosis as well [23]. Dynamical interactions have a spatiotemporal fluctuation which also has a scaling behavior. Homeostatic time-fluctuation is the so-called pink noise [24], that characterizes the noise of homeostasis.

The above complex biological processes connect to the biological allometry, scaling, non-equilibrium, and non-linear thermodynamics. Special self-similarity characterizes the mass-allometry by universal scaling, and it appears in a large category of living structures and processes [25], which rigorously optimizes the metabolic power in a universal frame, [26]. Scaling is a simple power function, (like $P(x)=ax^b$), where a and b are constants, therefore the form of $P(x)$ remains the same during any magnification of x. This scaling condition characterizes the biomaterials, which is indeed scaled universally on a very wide range of magnifications from the subcellular energy-consumption through mitochondria and respiratory complexes to the largest animals by scaling exponent α = 3/4, [27]. The fingerprint of complexity can be found in various fields of biology, showing unified principles of self-organization [28]. Note, that mitochondria probably has a key-role in this complex behavior of living objects, because the non-mitochondrial respiration scaling factor is lower, (α = 2/3), characterizing the simple surface-volume ratio in these processes, [29], however the robust category of living systems is scaled by complex manner [30]. Different conditions modify the power function [31], forming various universality classes by self-similarity.

Self-organized processes are widely investigated in solid-state reactions (precipitations, phase-transitions, aggregations, nucleation, growth, etc.). The theory of phase-transition involving simultaneous random nucleation and growth was pioneered by Kolmogorov [32], Johnson, Mehl [33] and Avrami [34]. It is called Johnson-Mehl-Avrami-Kolmogorov (JMAK) model, revised later by others, [35], [36]. It describes the kinetics of phase transformation when nucleation is spatially random. The JMAK theory and one of its formulation called Avrami-function (AF) were introduced for solids to serve as mathematical models of different biological processes, [37] [38] and even for DNA replication process, [39] too. Experimental data [40] [41] [42] [43], prove a certain universality of the Avrami-equation to describe the real processes, which could be a useful tool for further research, [44]. It is generally useful for studying different processes with no known special system parameters, similarly to the critical phenomena of the physical-laws near to the phase-transition [45].

The AF ($A(t)$) [46] in its most applicable forms:

$$\begin{aligned} &\ln\left(-\ln\left(A(t)\right)\right)=n\ln(t)+\ln\kappa \\ &A(t)=1-\exp\left(\kappa t^n\right) \end{aligned} \tag{1}$$

where t is the elapsed time of the process, κ depends linearly on the nucleation rate and on the growth-rate by the power of three. The so called "Avrami constant" (n) was introduced in simple model n = 4, and so originally in solids it was considered an integer [47]. It is interesting, that the space-fractal dimension

dependens on AF [48]. Here n value is not necessarily an integer and depends well on the processes that are described by it. The fractal dimension, and the power-law of self-similarity are tightly connected [49]. Experimental data show, that the progression of many reactions in biology also follow the $A(t)$ AF with various, non-integer characteristic constants [40]. It was observed universally in different processes from a wide range of structural and dynamical situations of living systems [44] [50] [51] [52].

The non-equilibrium thermodynamical formalism could be applied to a self-organized system of malignancy in space and time [53]. Cancer breaks the network of normal cells, while the cooperative tissue harmony changed to non-co-operative competitiveness forms a new complex structure non-linearly far from the thermodynamic equilibrium. Cancer could be described as a dynamical phase transition from healthy to cancerous [54], described with a clear analogy with phase transitions in a lifeless nature. Starting with an avascular situation and forming a dormant microscopic cluster [55], it continues to develop new angiogenetic formations by epithelial-mesenchymal cell transition, induced by bio-electromagnetic forces, [56]. Tumor leaves the dormant state by an allometric transformation [57], and the previously almost undetectable phase becomes traceable. An Avrami-like function in time describes its development [58]. This idea was used to show the validity of Avrami description [59] and extended to metastases while studying the transition of avascular appearance of tumorous clusters [60] to vascular phase, which bases the dissemination of malignant cells, [61]. Metastases are developed by a first order phase transition of cells from non-cancerous to metastatic ones [62]. The development of this new phase needs a great amount of energy. The energy dissipates in the system, produces a high rate of entropy development.

The general transport structure (blood-vessel network) of the tissues forms fractals by allometric scaling, including the angiogenetic processes in tumor formation [57]. In oncological applications, the available metabolic transport and the fractal dimensions of the angiogenetic network determine the average survival of a tumor. The average survival of the tumor-cells shortens by the growing fractal dimension of the transport network and modified by some kind of an alimentation of the tumor, [63]. The tumor-growth follows the universal law of scaling [64], which can be used in cancer-research [65].

The dynamics of the evolution of cancer produces various phases of the growing structures due to the genetic instability, leading to phase transitions [66]. Tumor development operates near the threshold of phase transition, destabilizing the actual structure, making it highly heterogeneous [67], producing a large variety of random mutations [68], finding the most optimal conditions of the further proliferation. Their development is based on competition, a "fight" for the individual survival. The optimal strategy is well known in the game-theory [69] where the mixed-strategy forms Nash equilibrium in the non-cooperative game by random variation behind [70]. This situation is typical for topological phase transi-

tions [71], where the cooperation emerges despite the selfish, non-cooperative individual participating cells [72].

Our objective in this article is to find a parametric description of overall survival, which fits the self-organized processes and able to show the inherent information of survival measurements of cancer patients.

2. Method

Most of the survival analyses in medical evaluations use the Kaplan-Meier (KM) non-parametric estimator [73] [74], used for incomplete observations. KM is useful to examine the probability of lifetime and effectivity of the chosen treatment for such lethal diseases like cancer. The computed probability of an event in a definite point of time:

$$\begin{pmatrix}\text{Probability at actual}\\ \text{time of observation}\end{pmatrix} = \frac{\begin{pmatrix}\text{Number of participants living}\\ \text{at the strat of observation}\end{pmatrix} - \begin{pmatrix}\text{Number of participants died or}\\ \text{censored during the of observation}\end{pmatrix}}{\begin{pmatrix}\text{Number of participants living}\\ \text{at the strat of observation}\end{pmatrix}}$$

KM estimator is defined by multiplying the above described successive probabilities by any earlier point of time obtaining the final estimate:

$$KM\left(t\right) = \prod_{t_i \le t}\left(1 - \frac{d_i}{n_i}\right) \tag{2}$$

where d_i is the number of deaths at the time t_i; t_i is a time when at least one death had happened in the examined cohort, and n_i is the number of individuals known to survive (not censored, exists in the study) at time t_i. Some modifications were done in tails (pessimistic approach when short-tailed) [75], and optimistic approach, a fat-tailed [76] is in use having a difference in survivals at the end of the trial.

The best method for mining data could be when the non-parametric KM survival plot can be parameterized. The description of survival curves by parametric distribution function is a long-term effort [77] allowing the optimization of the information from the measured dataset. For the correct parametrization, we have to take an overview on the scientific facts that we can use for the research of the optimal parametrization. The most important result available is the parametric solution that is connected to the spatiotemporal self-organization and the self-similarity of developed structures.

The parametrization of survival measures we use to the universality of life consideres its self-organized self-similarity. The progression of life involves nonlinear and non-equilibrium thermodynamical consequences including the fractal description and similar processes of the phase transitions in non-living systems. For calculating the survival-time, let T be the stochastic variable defined on the set of individuals, (lifetime). The lifetime distribution function is the probability

of the lifetime being less than or equal to t, namely

$$p_L(t) = P\{T \le t\} \tag{3}$$

Thus, the survival probability distribution (survival function) can be defined by the probability of the T lifetime being higher than t, that can be expressed in the form of

$$p_S(t) = 1 - p_L(t) = P\{T > t\} \tag{4}$$

The density function of the lifetime distribution function is the

$$f(t) = \frac{\mathrm{d}p_L(t)}{\mathrm{d}t} \tag{5}$$

probable density, therefore, the average lifetime is:

$$\langle T \rangle = \int_0^\infty t f(t)\mathrm{d}t = \int_0^\infty p_S(t)\mathrm{d}t \tag{6}$$

Introducing the $h(t)\mathrm{d}t$ death rate is the probability that in case of a t length survival time, death occurs at (t + Δt) and ($h(t)$ is the "hazard function" or "death rate"). Therefore, the probability is that in the case of a t length time survival, death occurs at ($t + \Delta t$) is

$$h(t)\Delta t = 1 - \frac{p_S(t+\Delta t)}{p_S(t)} = -\frac{\mathrm{d}\dfrac{p_S(t)}{\mathrm{d}t}}{p_S(t)}\Delta t = -\frac{\dfrac{\mathrm{d}\left[1-p_L(t)\right]}{\mathrm{d}t}}{p_S(t)}\Delta t = \frac{f(t)}{p_S(t)}\Delta t \tag{7}$$

From this:

$$h(t) = -\frac{\mathrm{d}\dfrac{p_S(t)}{\mathrm{d}t}}{p_S(t)} = \frac{f(t)}{p_S(t)} \tag{8}$$

It's cumulative form is

$$H(t) = \int_0^t h(\tau)\mathrm{d}\tau = -\ln\left(p_S(t)\right) \tag{9}$$

or

$$p_S(t) = \mathrm{e}^{-H(t)} \tag{10}$$

Biological systems are strictly self-organized [78]. The inherent property of the living objects is the self-organizing and the consequent self-similarity of the living structures [11], which could be the basis of the proper parameterization of survival.

Taking the self-similarity into consideration, death-rate (failure rate in (8)) must be a self-similar time function [44], mirrored by a scaling like:

$$h(t) = \alpha t^\beta \tag{11}$$

Its self-similarity is obvious because it gives the same function by magnification m:

$$h(mt) = \alpha(mt)^\beta = m^\beta \alpha t^\beta = m^\beta h(t) \tag{12}$$

The survival probability distribution function from (9) and (10) is:

$$p_S(t) = \mathrm{e}^{-\int_0^t h(\tau)\mathrm{d}\tau} \tag{13}$$

The self-similar death rate (hazard function) is:

$$H(t) = \int_0^t \alpha\tau^{\beta}\mathrm{d}\tau = \frac{\alpha}{\beta+1}t^{\beta+1} \tag{14}$$

Substituting (14) with survival (13), we get:

$$p_S(t) = \mathrm{e}^{-\int_0^t \alpha\tau^{\beta}\mathrm{d}\tau} = \mathrm{e}^{-\frac{\alpha}{\beta+1}t^{\beta+1}} \tag{15}$$

Introducing

$$t_0 = \left(\frac{n}{\alpha}\right)^{1/n} \quad \text{and} \quad n = \beta+1 \tag{16}$$

Hence:

$$p_S(t) = \mathrm{e}^{-\left(\frac{t}{t_0}\right)^n} \tag{17}$$

which has two parameters for one curve, t_0, is the scale parameter, which is the natural scale of the time-function variation, and n is the shape parameter. Consequently, the lifetime distribution function $p_L(t)$, by (3) and (4) is the well-known AF ($A(t)$) or cumulative form of the two-parametric cumulative Weibull distribution ($W(t)$):

$$p_L(t) = A(t) = W(t) = 1 - \mathrm{e}^{-\left(\frac{t}{t_0}\right)^n} = 1 - p_S(t) \tag{18}$$

with additional conditions $t \geq 0$, $A(t) = W(t) = 0$, when $t < 0$. The inverse function, when the t-time is calculated from a given p probability is:

$$t = W_{inv}(p) = t_0\left(-\ln(1-p)\right)^{1/n} \tag{19}$$

There are various parameters characterizing the WF from the time of development independently. The shape parameter of WF is usually $n > 1$, following a sigmoid curve, which form is a psychometric function [79] anyway. In cases when $n \leq 1$ the survival is a simple exponential function with rapid decrease by the decreasing of n.

The cumulative Weibull distribution (Weibull function, WF) is highly universal and represents all the features described in the introduction above. The formal identity of WF with the AF in JMAK inherently involves the phase transition approach, and the mechanics follow the tumor kinetics, [59].

The AF and WF have been used for a long time for survival/reliability description. Originally Weibull's statistics was developed to describe the fracture of brittle materials [80], [81] and to calculate the probability of the damage-free survival of the given material. It can be derived from geometric scale invariance (fractal organized structures) by physical principles, [82] in mechanical mills. It is frequently applied in the study of mechanical fatigue and failure [83].

The fit of WF to the non-parametric KM is completely rigorous when a strictly homogeneous cohort of patients is investigated, with unified equivalence of the participating individuals followed until the decease or censoring. This grouping selection apparently limits the applicability of WF. The parametrization of the aging and natural death has no such grouping selection, it is related to every human being and their survival. The epidemiological studies in gerontology refer to the Gompertz-distribution, [84]. The Gompertz function (GF) is a function of time. When $G(t)$ represents the number of individuals in the given period of time, t, G_0 is the number of subjects at the start of the counting time, then GF is:

$$G(t) = G_0 \exp\left(-a \cdot \left(\exp(b \cdot t) - 1\right)\right) \tag{20}$$

The parameters a and b are positive and a is connected to the growth, while b is connected to the displacement in variable t. GF is also a double-parametric function, similarly to the n and t_0 in WF.

During the historical development of WF, it has started to characterize the aging of the non-living components and machineries (reliability) while the GF was initially developed for the ageing of living objects [85]. By developing the statistical methods, soon, both the Weibull and Gompertz distribution have started to be applied for description of tumor-development and cancer-death. The comparison of the two distributions shows that the best fit of GF is ($a = 9.03$, $b = 2.58$) and the best fit of WF is ($n = 1.11$, $t_0 = 0.04$); ($1 - r^2 = 3.6 \times 10^{-6}$, SE = 0.002); where SE is the standard error of the regression estimate minimizing the sum of squares of measured and estimated data-pairs. Due to their applicability, the Gompertz and Weibull distributions are both commonly used in biological and engineering reliability investigations [86], [87].

The study of Gompertzian distribution for tumors supports a hypothesis that the fractal structure weakens and, in the end, it disappears by the growth of the tumor [88]. In general, the tumor-growth follows a universality, [64] [89], which prefers to use the WF. The clear fitting of allometric scaling by the fractal structure of the tumor [64] shows not only the tumor growth but the validity of the allometry in the growth of the axillary lymph node involvement in breast cancer [90]. In consequence, we choose to use the WF for modelling the KM plot of the overall survival.

The Gompertz distribution could be obtained by the reduction of the generalized exponential Weibull distribution [91], which formulated in a more general form, proposing to derive both distribution from one single [92] and it is applied for survival data with pretty good results.

The GF does not satisfies the self-similarity (formulated in (11)), and therefore, it is not in harmony with self-organizing biological dynamics, which is a certain character of the harmonized biological development, [2]. This might be the reason, why the WF describes the intrinsic causes of age-related mortality

better (following the homeostasis in the healthy aging process) while the Gompertz distribution reflects the extrinsic factors [93]. Due to the self-similarity of WF, we expect, that the self-organized biological development of tumors intrinsically developing in a healthy environment from where it derives, prefers the WF to describe the KM in malignant diseases accurately. It is a further support for the primary importance of Weibull distribution, that it is derived from the ontological law, and so it is directly connected to the self-organized structure of the living matter [28]. The self-similarity, as the basic fingerprint of self-organizing is not valid in Gompertz distribution. The "mystery" of Gompertz function is probably the equilibrium between the predictable and unpredictable (chaotic) dynamisms, [94]. Contrary to the exponential origin of GF, the self-similarity (power function) of WF's origin hypothesizes some parallels with the opposite pictures of fractal-like organizations and general scale-free (small-words, [95]) large networks (exponential function). Despite the structural preference of WF, GF also fits well to allometry, represented by power-function [96], shown in the development of rats [97]. Although WF fits very well to the growth function of the general ontogenic model, using the data for rat [98] ($r^2 = 0.99965$, $\mathrm{SE} = 0.949$); the fit of GF shows the same result ($r^2 = 0.99967$, $\mathrm{SE} = 0.884$) for the same allometric curve. The difference is negligible in this regime of development. In the case of animals with larger masses, the difference is also not significant. It is subtle, favoring only the WF for the description of the best regression fit to the allometric scaling result, using the available data from [98]. (The best WF and GF fits to allometry for cow are ($r^2 = 0.99978$, $\mathrm{SE} = 1.021$) and ($r^2 = 0.99972$, $r^2 = 0.99972$), respectively.)

WF is successfully applied to the living processes as the psychological function [99], describing the sensing processes well in connection with Weber-Fechner law [100], establishing psychometry [101]. Lifetime estimations are frequently approached by WF [86] and WF is also successfully used for clustering gene expression [102].

WF describes the non-parametric KM plot with appropriate accuracy in gerontology [103] [104]. A mathematical link of natural death-rate, aging and complexity is a fundamental tool of lifetime estimation [105] [106], using time-dependent shape-factor ($n \cong 7/\left|\ln\left(t/t_0\right)\right|$) to describe the natural death at the end of life. Cancer-death was also described by WF with time-dependent shape-factor, using a similarity between the fracture survival of brittle materials and the specific survival characteristics of a cohort of cancer patients [107] [108]. In this model the shape factor linearly depends on the time and gives surprisingly accurate fit to the data from the cancer-registers.

Due to its self-similar behavior, fractals could be used for modeling cancer [109], and the KM survival plot divided significantly by fractal dimension shows the prognostic value of the fractal analysis well [110]. Consequently, it is possible to evaluate the various images in oncology by the fractal structure and these images can be characterized by Weibull distribution as well [111].

Due to the self-similarity, the parametric distribution generally fits well with the KM plot, and so it is successfully used in oncology [112] [113]. The application of the parametric WF approximating the survival curve is a standard approach for the evaluation of clinical trial data, and so it is established theoretically and practically, [34] [86] [99] [114]. Comparing various parametric fits to KM survival plot, the WF was the most accurate [115]. The model was used to analyze the prognostic factors of the survival of cancer patients, and it was proved in a large retrospective analysis with $n = 746$ gastric cancer cases, [116].

Summarizing the above, the self-organizing and the self-similarity are universal laws fingerprinted in the fractal description and can be described by cumulative Weibull distribution. This universality of WF is applied to parametrize the KM plot. Due to the universality, the WF parametric regression fits the KM plot with sufficient accuracy and so determines the KM curve by two parameters (t_0 and n). On the regression, a considerable improvement could be made by smoothing the KM with the hazard data (patients at risk), [117]. Other improvements of the bivariant fit are also available [118], but for simplicity we use the original WF fit to KM insisting on showing the roots that are the universality of WF in survival investigations. Further smoothing and corrections are additional to the clearly established basis, due to the deviations in real cases.

3. Results

The characterization of WF has four special points, the value at t_0, the mean, the median and the inflection point. The median, the mean and the mode (the maximum point in the distribution function is an inflection point in the cumulative curve) are calculable from the parametric formulas, (see **Figure 1**):

$$\begin{aligned} &\text{median}\left[p_S(t)\right] = t_0\left[\ln(2)\right]^{\frac{1}{n}} \\ &\text{mean}\left[p_S(t)\right] = t_0\int_0^{\infty} \mathrm{e}^{-x} x^{\frac{1}{n}}\mathrm{d}x = t_0\Gamma\left(1+\frac{1}{n}\right) \\ &\text{mode}\left[p_S(t)\right] = t_0\left[\frac{n-1}{n}\right]^{\frac{1}{n}} \end{aligned} \tag{21}$$

The corresponding probabilities when $t_0 = 1$ and $n = 2$, are 0.5, 0.607 and 0.456 for the median, mode and mean, respectively. The quantile of this function is ≈0.632 and it independent from n value. Limit $\lim_{n\to 0} p_S(t) = 0$ through a step-function at $t = 0$, while $\lim_{n\to\infty} p_S(t)$ is a step function at $t = t_0$, (**Figure 2**). All the noteworthy points are proportional to t_0, so the natural units of the elapsed time are $t_0 = 1$, when the single n-parameter defines the function. The hazard function (9) is constant when $n = 1$ (or $\beta = 0$, which means the parameter has no effect on the hazard), and it is increasing and decreasing when $n > 1$ (meaning the event is more likely to occur) and $n < 1$, (meaning the event is less likely to occur), respectively. The limit $\lim_{n\to 0} H(t) = 0$ is a step-function at $t = 0$, and $\lim_{n\to\infty} H(t)$ is a step function at $t = t_0$, (**Figure 2**).

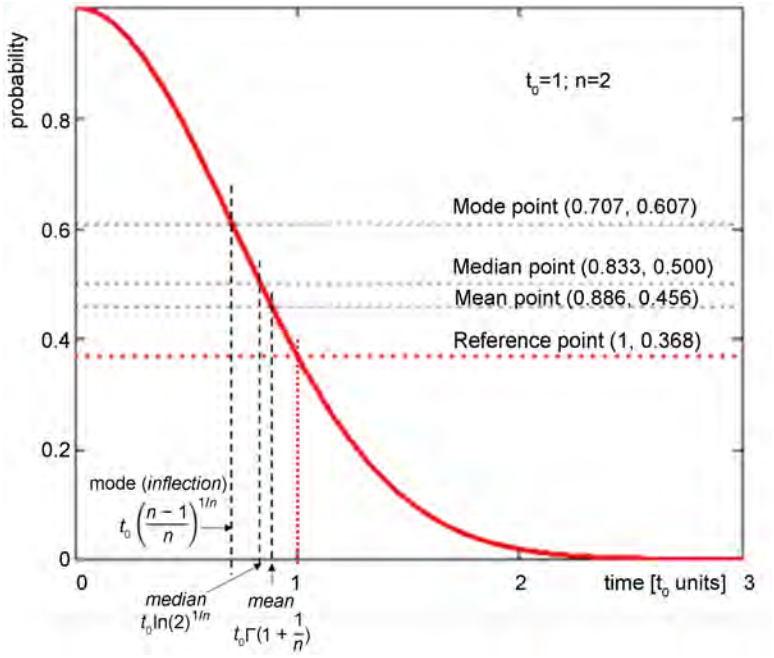


Figure 1. The noteworthy points of the Avrami-Weibull function, when $t_0 = 1$ and $n = 2$. The reference point of the Avrami-Weibull function is the value (1/e ≈ 0.37), where $t = t_0$. The inflection point marks the mode of the distribution, which is the most frequent probability. When $t_0 = 1$ is chosen, it will be the unit of the elapsed time.

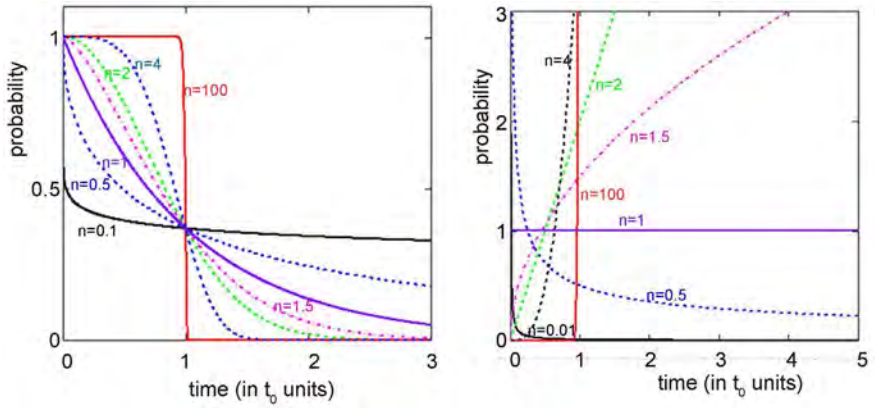
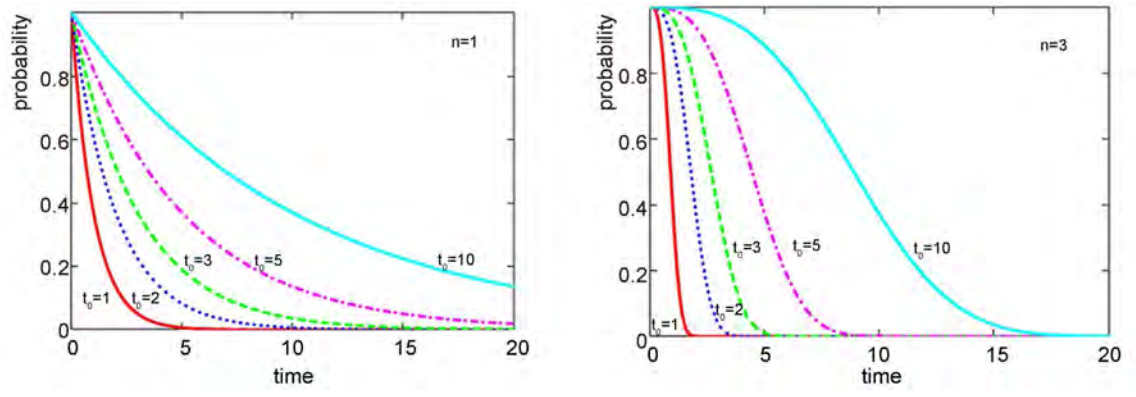


Figure 2. The limits of (a) survival ($p_S(t)$) and (b) hazard ($H(t)$) functions ($t_0 = 1$).

The various parameter-pairs of WF are shown in **Figure 3**.

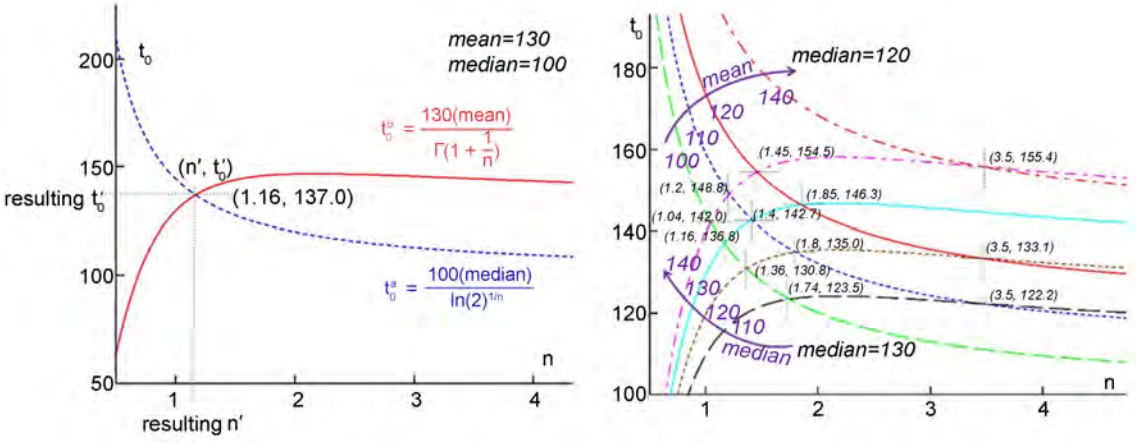
The inflection point in the WF (cumulative Weibull distribution) is the mode of the probability distribution function. It is the most likely appearing value in the Weibull probability distribution function. The inflection in the WF of survival divides the speed of developing death, which reaches its maximum at this point and the transfer of inflection is slowed by the elapsed time.

Programming calculates the result or makes it graphical (**Figure 4**). This makes it possible to generate the Weibull fit for the Kaplan-Meier routinely by knowing its median and mean values.



(a) (b)

Figure 3. WF with various parameters (a) changing t_0 (scale parameter) at constant $n = 1$ (shape parameter); (b) $n = 3$.



(a) (b)

Figure 4. Graphical solution of reestablishing the Weibull parametric survival curve from the median and mean values. (a) example: t_0^a and t_0^b are the time curves from median and mean expressions, respectively. Their common point (crossing) gives the t_0 and n parameters of the WF, which—in this case is when the mean is 130 and median is 100, the $t_0' = 137$ and $n' = 1.16$; therefore, it looks like this: $W(t) = e^{-\left(\frac{t}{t_0}\right)^n} = e^{-\left(\frac{t}{137}\right)^{1.16}}$. (b) a few solutions to show the trend of the graphical results.

The data at the particular points vs. n are shown in (**Figure 5**). The mode changes rapidly in the interval of n (1, 2), so reading accurately is difficult, therefore the median and mean are proposed to reestablish the entire WF. However, in a value of $n \approx 3.35$ at $t_0 = 1$ the values of mode, mean and median are practically identical, so the WF could be characterized with a single parameter. Increasing t_0 does not lead to a significant change of the situation, so in virtually every case, we may approach WF only with one parameter over $n \approx 3.35$.

In conclusion from the above, the parametric regression KM is universally determined by two parameters (the shape parameter (n) and the scale parameter (t_0) of WF), due to the basic behaviors of living processes: their self-organizing

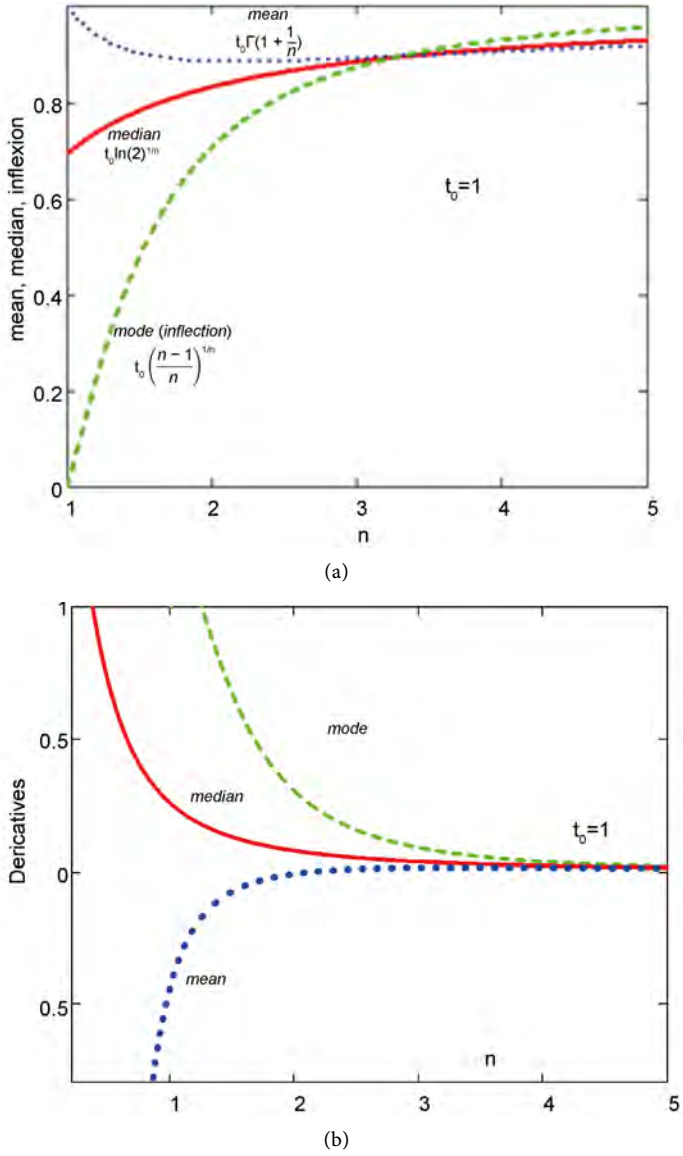


Figure 5. Characteristics by shape-parameters at $t_0 = 1$. (a) function vs. n; (b) derivatives vs. n.

and self-similarity, which is characterized well by their spatio-temporal fractal structure. When a clinician tries to describe the main info of the KM survival curves, takes the median value of survival, as a significant parameter characterizing the actual survival result into account. This is, in fact, an automatic characterization by a single parameter of the non-parametric estimation. However, the median alone cannot characterize the long tail of the KM plot; it does not consider the history of the patients in the remaining second half of the cohort, which could be essential for measuring the "cured" [119] anyway. Studying the median alone disregards the real measurable success at the end of the study. Correcting this "mistake" the average (mean) of the KM non-parametric distribution is considered. The mean is affected more by the "tail" of the distribution, so it gives a more accurate idea on the cure rate. The median is more responsible

for the information about the rapidity of the loss of the patients, while the mean has more part in the information about the length of the effect of the high-success patients, Figure 6.

Sometimes the inflection of KM is studied too, having the highest death-rate in the study at that point. All are important for characterization, but two of them are independent, and the third could be calculated from the chosen two. The distribution curve must be characterized by two parameters at least.

Two of the three noteworthy points (median, mean, inflection) of the KM may parametrize the non-parametric plot. Measuring or guessing these characteristic points (mainly the median and mean) is a standard comparison of the KM-plots and usually accepted as the result of the actual study. These points really characterize the non-parametric distribution and give the possibility to parametrize, so, in fact, this is a "hidden" parameterization of the KM plot by WF.

A simple approach of Weibull fit could be made on the KM plot by its derivative in the t_0 reference point, which is proportional to $-n$. (The derivative there is exactly $\frac{\mathrm{d}W(t_0)}{\mathrm{d}t} = -\left(\frac{1}{\mathrm{e}}\right)\frac{n}{t_0} \cong -0.368\frac{n}{t_0}$.) Therefore, the parametric evaluation could be checked well at the $t = t_0$ point, and the complete parametrization could be established approximately by the value of the t_0 point and the value of its slope, Figure 7.

The regression could be simplified to linear by double logarithmic approach:

$$\ln\left[-\ln\left(W(t)\right)\right] = n\ln\left(\frac{t}{t_0}\right) = n\ln(t) - n\ln(t_0) \tag{22}$$

The regression is shown in Figure 8. Note, that this approach is less precise than the function fit, because the double logarithm suppresses the accuracy in real KM fit.

However, the obvious deviation of the regressions from the measured OS is in

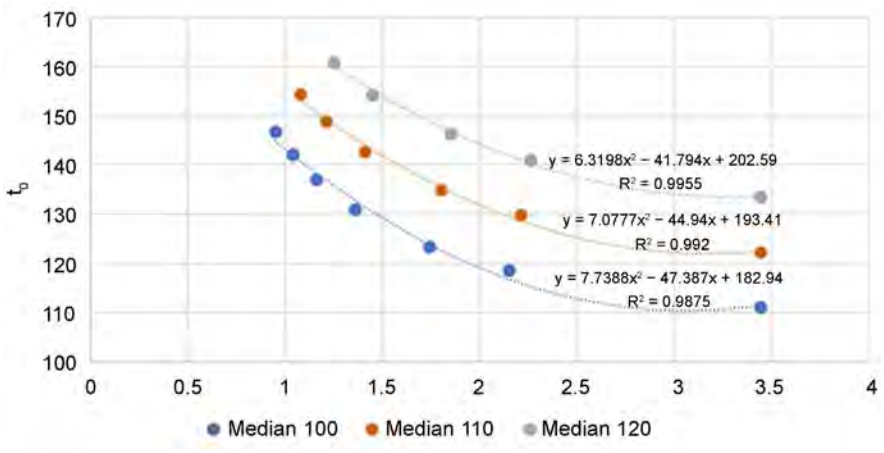


Figure 6. The mean and median changes according to the n and t_0 parameters. Parabola fits rather well, which connects the two parameters (n and t_0) at different medians and means.

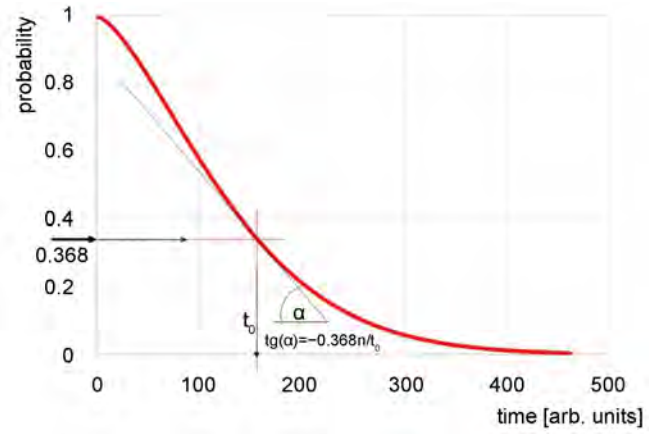


Figure 7. A quick check of the parameters of the Weibull-fit to KM. real process on a KM ($n = 1.5$, $t_0 = 150$).

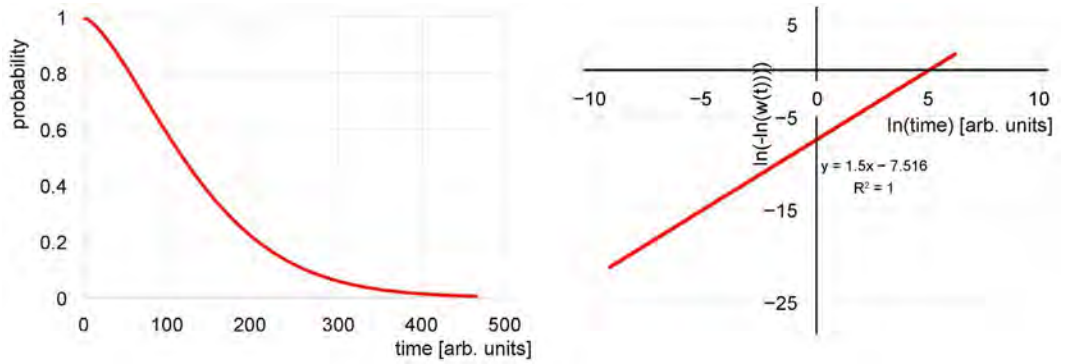


Figure 8. Logarithmic determination of the Weibull parameters ($n = 1.5$, $t_0 = 150$). (a) original WF, (b) $\ln\left[-\ln\left(W(t)\right)\right]$ vs. $\ln(t)$.

the tail of KM, which is similarly not followed by both functions. The universal WF idea offers regression fit to the KM for a group of patients who have had an event or have censored until the end of the study. This is, of course, limited in real trials. We consider any chosen cohorts inhomogeneous because of the huge variability of living conditions. A homogenous group of patients, which has identical individuals could never be selected. However, there is a possibility to divide the cohort to subgroups with very similar patients, and fit WF on these independently, while the measured KM is, of course, a sum of the results of all the subgroups. With M subgroups in the complete cohort of N patients, and every group containing $k_1, k_2, \cdots, k_M$ patients, the WF for the actual measured non-parametric KM will be:

$$W^{(KM)}(t) = \frac{k_1}{N} e^{-\left(\frac{t}{t_0^{(1)}}\right)^{n^{(1)}}} + \frac{k_2}{N} e^{-\left(\frac{t}{t_0^{(2)}}\right)^{n^{(2)}}} + \cdots + \frac{k_M}{N} e^{-\left(\frac{t}{t_0^{(M)}}\right)^{n^{(M)}}} \quad (23)$$

$$\text{or } W^{(KM)}(t) = \sum_{i=1}^{M} \frac{k_i}{N} e^{-\left(\frac{t}{t_0^{(i)}}\right)^{n^{(i)}}} \quad \text{and} \quad \sum_{i=1}^{M} k_i = N$$

By taking extra care to have a homogeneous cohort, at least the time-limit of the study forms a group from patients, who had no event (or are not censored). The "remaining" patients in the given treatment study have the highest benefit from the performed treatment or they were in a definitely different condition when they were selected into the cohort. We call this group "remained group" (RG) due to the lack of proof of complete recovery. However, this group is sometimes regarded (incorrectly) as a cured fraction (according to the endpoints of the study). In a rigorous approach the disease-free survival (DFS) has to be compared with the matched healthy control group, and the cure-rate on this comparison must be decided [120]. An alternative way to determine the group of "cured" patients and the connected value of the "cure" time is when the hazard rate of the studied group corresponds to the hazard in the general population [121]. When it fits, we may talk about the real cure rate, which does not mean that an event cannot happen due to independent reasons from the investigated disease.

The KM curve in an RG situation obviously does not fit to the strict WF, which must be decreased to a zero cumulative probability. When the ratio of the remaining individuals is $c_{RG} = n^{RG}/N$, the KM plot can be approximated with reasonable accuracy by the weighted sum of two WFs. In the RG fraction, the time-parameter is longer than in the fraction of patients having an event or censored.

$$W^{(c)}(t) = (1-c_{RG})\mathrm{e}^{-\left(\frac{t}{t_0}\right)^n} + c_{RG}\mathrm{e}^{-\left(\frac{t}{t_0^{(RG)}}\right)^{n^{(RG)}}} \tag{24}$$

In this case, the composition of the time-parameter of the long survival WF fit is practically infinite (compared to the time-length of the study):

$$W^{(RG)}(t) = \mathrm{e}^{-\left(\frac{t}{t_0^{(RG)}}\right)^{n^{(RG)}}} \cong 1 \tag{25}$$

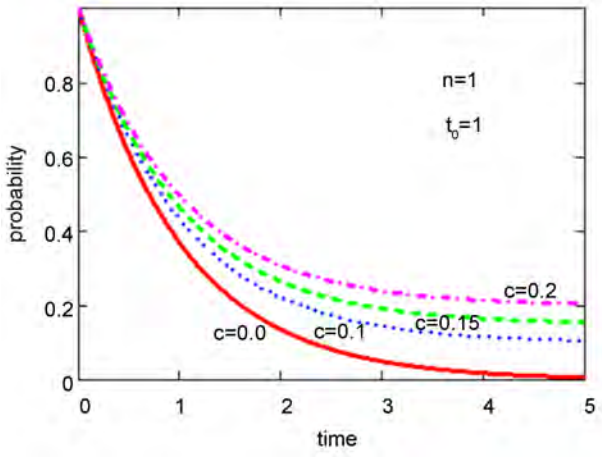
In this case, the correction by a survived fraction of the patients is constant. Denoting the constant correction *c*, the plot will be composed by this:

$$W^{(c)}(t) = (1-c)\mathrm{e}^{-\left(\frac{t}{t_0^{(c)}}\right)^{n^{(c)}}} + c \tag{26}$$

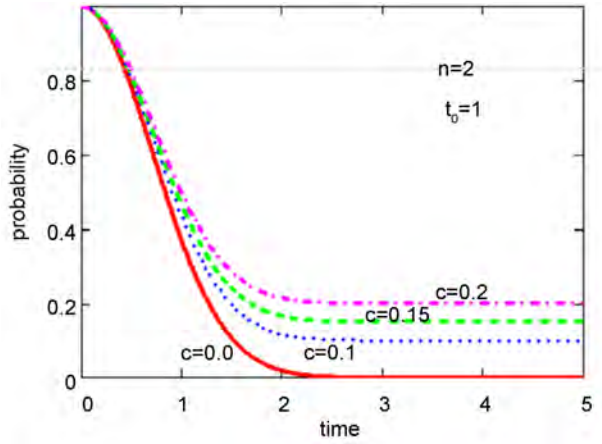
The variation of c shows different fitting functions, **Figure 9**:

Characterization of the curative effect of the treatment making a WF fit to the non-parametric KM survival could be done with the Shannon-entropy. Entropy measures the information carried by the probable density function (pdf, $p(t,n,t_0)$) behind the WF ($W(t,n,t_0)$). It measures the probability of realization of an event or censoring

$$p(t,n,t_0) = \frac{\mathrm{d}W(t,n,t_0)}{\mathrm{d}t} = \frac{n}{t_0}\left(\frac{t}{t_0}\right)^{n-1}\exp\left(-\left(\frac{t}{t_0}\right)^n\right);$$
$$\int_0^\infty p(t,n,t_0)\mathrm{d}t = 1 \tag{27}$$



(a)



(b)

Figure 9. The WF with various c concentration of the patients in the RG group.

The quantity of information is $I(t,n,t_0) = -\ln\left(p(t,n,t_0)\right)$ which is realized by $p(t,n,t_0)$, so the complete information from the system is the classical Shannon-entropy, [122] is:

$$S_{Sh}(n,t_0) = -\int_0^\infty p(t,n,t_0)\ln\left(p(t,n,t_0)\right)\mathrm{d}t \tag{28}$$

A higher entropy shows less information (more uncertainty). When an event has a lower probability to occur, it carries more information, so its Shannon-entropy is lower than the effects of the frequent occurrence. The expectation of a random variable is characterized by this entropy, so by this meaning it is a direct analog for the entropy definition in physics (statistical thermodynamics). When the informational entropy decreases, (its change becomes negative) it means that the probability distribution differs from the uniformed distribution, concentrating to some data.

The entropy growth in physics usually happens when the system approaches equilibrium, while in pdf the increase of entropy shows a lack of information when the average rate of information produced by the stochastic source of the

data decreases.

The Shannon entropy (28) measures the diversity of probability distribution function (pdf) behind WF (in fact the derivative of WF). It is a sum of the n and t_0 dependent parts:

$$\begin{aligned} &S_{Sh}(n,t_0)=\gamma\left(1-\frac{1}{n}\right)+\ln\left(\frac{t_0}{n}\right)+1=S_{Sh1}(n)+S_{Sh2}(t_0)\\ &\text{where } S_{Sh1}(n)=\gamma\left(1-\frac{1}{n}\right)-\ln(n)+1;\ S_{Sh2}(t_0)=\ln(t_0) \end{aligned} \tag{29}$$

and γ is the Euler-Mascheroni constant: $\gamma\cong 0.577$ The special points of this entropy function are:

$$\begin{aligned} &S_{Sh2}(1)=0;\ \max\left(S_{Sh1}(n)\right)=S_{Sh1}(\gamma)\approx 1.127\\ &S_{Sh1}(0.173)\cong 0;\ S_{Sh1}(4.223)\cong 0;\ S_{Sh1}(0.363)\cong 1;\ S_{Sh1}(1)=1\\ &\lim_{n\to\infty,t_0\to\infty} S_{Sh}(n,t_0)=-(1+\gamma) \end{aligned} \tag{30}$$

The entropy (diversity) monotonically grows by t_0 in a logarithmic way, while it rapidly grows by n reaching the maximum at $n=\gamma$ (when $t_0=1$) and decreases from that point reaching zero at n = 4.223 (when $t_0=1$) and building information from that point (decreasing), so the step-function of WF (definite step) starts to dominate. The division of the entropy of a shape and scale (time) dependent part gives a possibility to define the role of these parameters. While the scale (time) parameter increases the Shannon-entropy monotonically, the shape parameter (n) after a maximum at γ, decreases the entropy, showing an increasing amount of information about the death (decreasing info about being alive) of the participants in the cohort. The growing shape-factor n definitely worsens the survival over the value γ, while the growth of the scale (time) factor gives longer survival expectations.

The Shannon entropy could be calculated real-time t ($S_{Sh}(n,t_0)$) and also could be relative to t_0 time, meaning, that the time is measured in t_0 units ($S_{Sh0}(n)=S_{Sh}(n,1)$), estimating the self-time. A higher entropy value means a higher uncertainty of death (therefore, a lower certainty of being alive). We expectthe growth of Shannon entropy of the parametric probability distribution function in cases of better results of the treatment.

4. Discussion

To demonstrate the parametrization, we use a large number of patients (1180 individuals), with various tumors treated by numerous standard therapies, but having one thing in common: they are treated by complementary modulated electro-hyperthermia (mEHT), when the standard treatment fail to deliver the desirable results, [123] [124]; **Figure 10**.

Using the approximate parametrization by the evaluation of this KM plot with the slope in t_0, we get $t_0\approx 43$ and $n\approx 0.9$. *median* ≈ 28, **Figure 11**.

The fit of single parametric WF curve to the KM plot, (**Figure 12**). The single

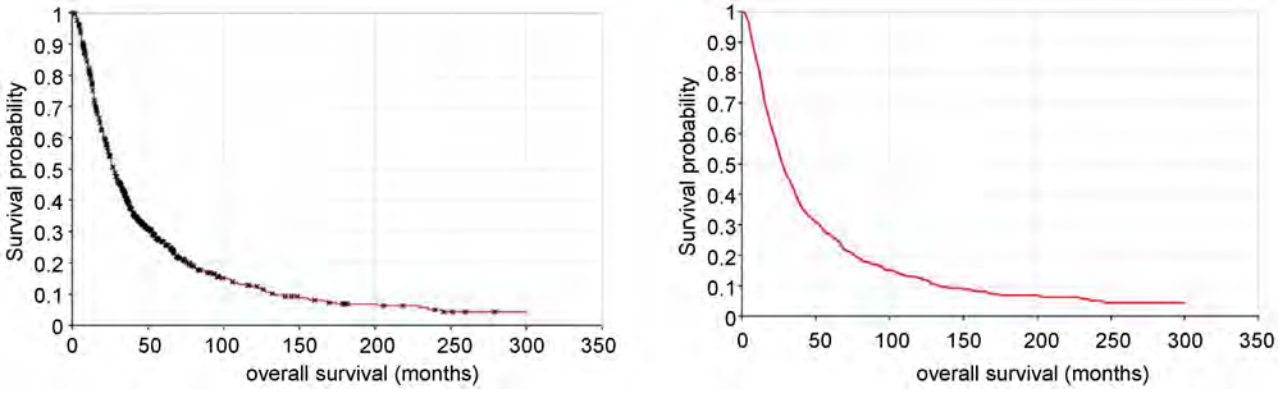


Figure 10. The overall survival KM-plot of a large number of patients (Pts.) = 1180 patients (various advanced solid malignancies, treated by complementary modulated electro-hyperthermia (mEHT), [117] [118]. The KM-plot contains very long (25 y) survival too. (a) with censored cases, (b) without censoring (for clarity).

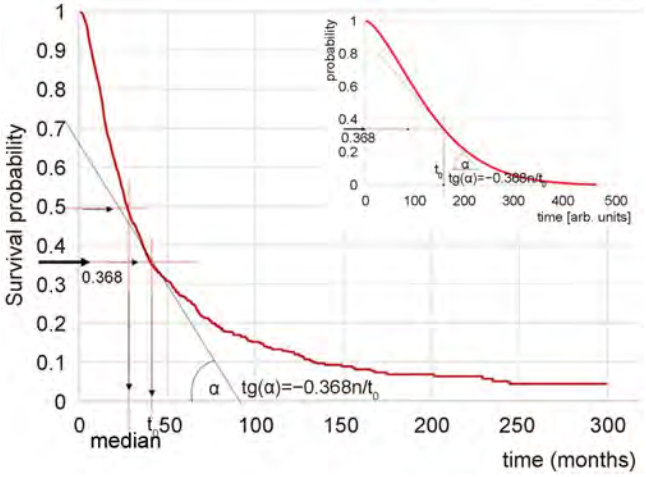
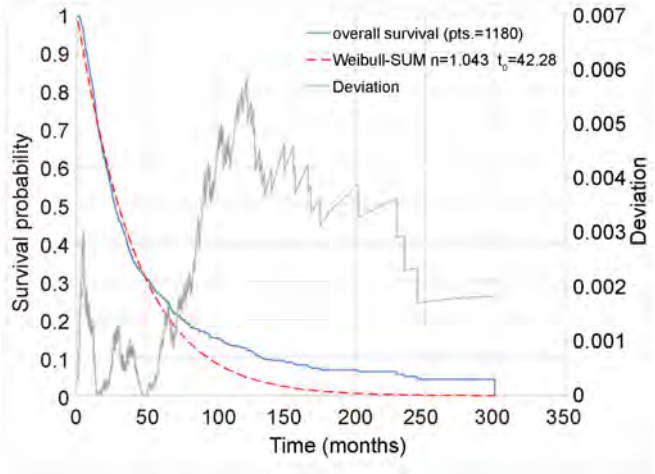
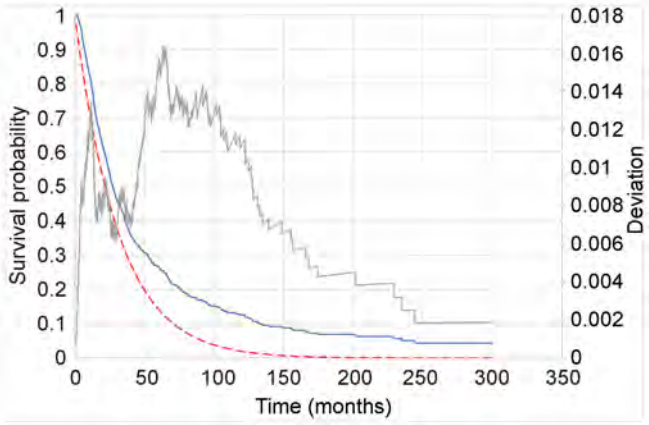


Figure 11. Rough determination of the parameters of the Weibull-fit to KM. Real process on a KM of Pts. = 1180 patients suffering in various malignant diseases [117], **Figure 10**. The obtained parameters are: $t_0 \approx 43$ and $\mathrm{tg}(\alpha) \approx 0.7/85 \approx 0.008$, hence $n \approx 0.9$. Control: median ≈ 28, which is approximately correct. (The principle of the process is in the insert in the figure).



(a)



(b)

Figure 12. WF fit (dashed line) to KM solid line to the overall survival KM-plot of pts. = 1180 patients (**Figure 10**) (a) Regression by deviation minimum SE = 1.6544; r^2 = 0.9850, n = 1.043; t_0 = 42.28; $S_{Sh} = 4.726$; (b) regression by correlation maximum. SE = 15.422; $r^2 = 0.9969$, $n = 1.013$, $t_0 = 31.142$; $S_{Sh} = 4.433$.

WF fits with an acceptable accuracy; the largest deviation is less than 0.007, (0.7%).

Note, that there is a difference, when we fit by minimalizing the deviation of the curves, $\mathrm{SE} = \min\left\{\sum_i \left(x_i^{KM} - x_i^{WF}\right)^2\right\}$ or

$r^2 = \left(\dfrac{\sum_i \left(x_i^{KM} - \left\langle x^{KM}\right\rangle\right)\left(x_i^{WF} - \left\langle x^{WF}\right\rangle\right)}{\sqrt{\sum_i \left(x_i^{KM} - \left\langle x^{KM}\right\rangle\right)}\sqrt{\sum_i \left(x_i^{WF} - \left\langle x^{WF}\right\rangle\right)}}\right)^2$ the square of Pearson correlation (where the $\langle\ \rangle$ bracket means the mean of the variable). The obvious difference is due to the different meaning of fit. The parameter SE minimizes the difference between the curves, while the r^2 minimizes the shape difference (maximizes the similarities) of the curves. A comparison with Shannon entropy shows more certainty (less uncertainty) by about 6% in the regression by minimizing SE than maximizing r^2. In the following, when we do not note the opposite, we use the minimal SE regression.

The fit is accurate, having no more difference in any compared points of the curves than 1%, but it is not accurate enough at the end of the observed time, due to the RG group of the patients. The deviation could be less with applying the RG principle of (26), **Figure 13**. The $S_{Sh} = 4.543$, which is 2.5% higher, mirrors the RG part of the patient distribution.

The parametric decomposition gives better fit by two WFs according to (24), **Figure 14**, where the r^2 has reduced drastically. The result shows the responding group (response rate (RR) 48%) and the non-responding one (52%). Note, that the less-responding group could be regarded as a non-responding control-arm.

The long-survival part of KM-plot has a higher entropy and shows more uncertainty of the death in both approaches. A better fit can be achieved when we

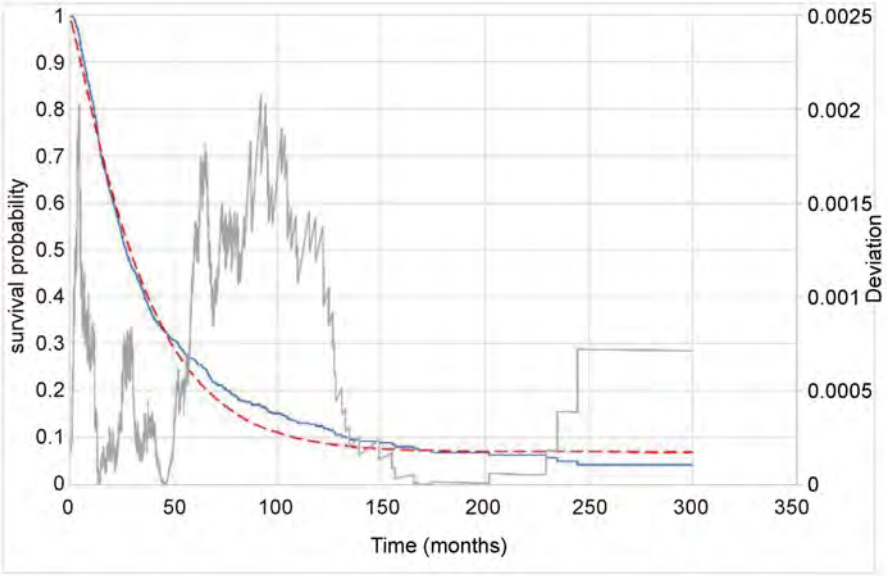
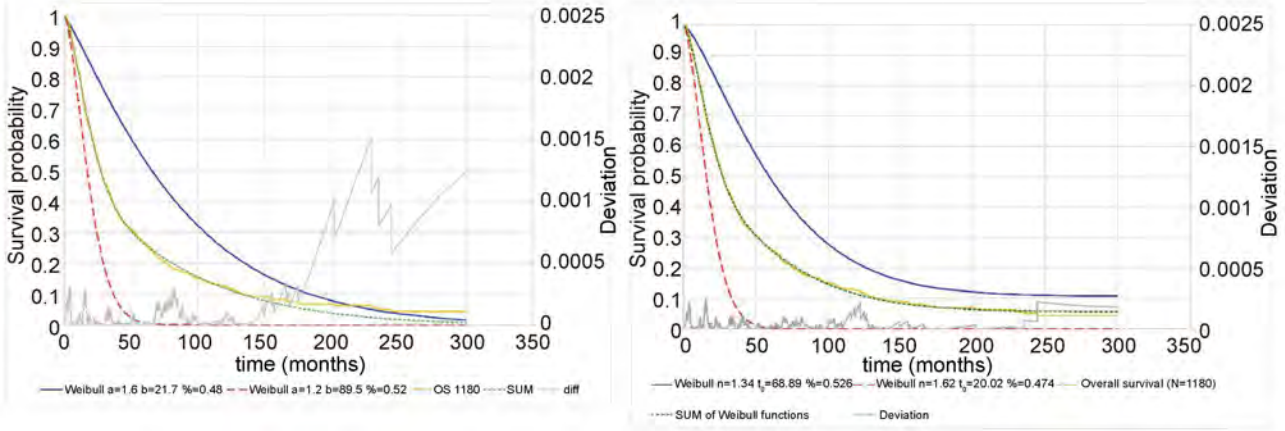


Figure 13. WF fit (dashed line) to KM solid line) from **Figure 10**. The applied RG is 7%. $\mathrm{SE}=0.9059$; $r^2=0.9918$, $n=1.135$, $t_0=36.632$; $S_{Sh}=4.543$. The largest square of deviation of the point-pairs (LD) is 0.002 (0.2%).



(a) (b)

Figure 14. (a) The double Weibull fit to the overall survival of $n=1180$ patients in malignant diseases complementary treated by the mEHT method. The longer survival (solid line) is the group of responding patients for the treatment; while the shorter survival time (dashed line) is a component of the composite fit of WF regarded as a non-responding group, that could be used as a reference cohort of patients. The sum of the two components (dotted line) fits to the measured overall survival. Deviation of the regression is shown in light solid line with values on the secondary axes. (a) Decomposition using both WF without RG ($\mathrm{RG}=0$, $\mathrm{RR}=48.4\%$, $\mathrm{SE}=0.0968$; $r^2=0.99954$); longer survival component ($n=1.17$, $t_0=89.52$; $S_{Sh}=5.423$); shorter survival component ($n=1.59$, $t_0=21.74$; $S_{Sh}=3.831$); (b) Decomposition using RG ($\mathrm{RG}=10.7\%$, $\mathrm{RR}=52.6\%$, $\mathrm{SE}=0.0596$; $r^2=0.99954$); longer survival component ($n=1.34$, $t_0=68.89$; $S_{Sh}=5.086$); shorter survival component ($n=1.62$, $t_0=20.02$; $S_{Sh}=3.734$). KM-plot and the sum of decomposed Weibull curves suppressed are remarkable (solid line) compare to the single fit (dotted line).

count RG. The RG is obtained from the remaining survival fraction in most of the actual cases, and it has measurably longer survival than the study follows the patients who had no event or were not censored earlier. RG is a part of the

"censored" patients at the end of the study.

For an easier calculation of the WF fractions (components) of the KM-plot, we may use the logarithmic evaluation of the survivals, which modifies the grouping more than the above decomposition. A linearly fit function $\ln\left[-\ln\left(W\left(t\right)\right)\right]$ by $\ln\left(t\right)$ of KM is shown in Figure 10. According to (22) it shows rather large deviations at the start and at the end of the curve, Figure 15.

The original WF fit shown in Figure 12(a), and the linear fit from the logarithmic approach of Figure 15. differs from each other, Figure 16. The deviation of the logarithmic fit is more than double in some intervals, so the direct fit of WF to KM is more accurate.

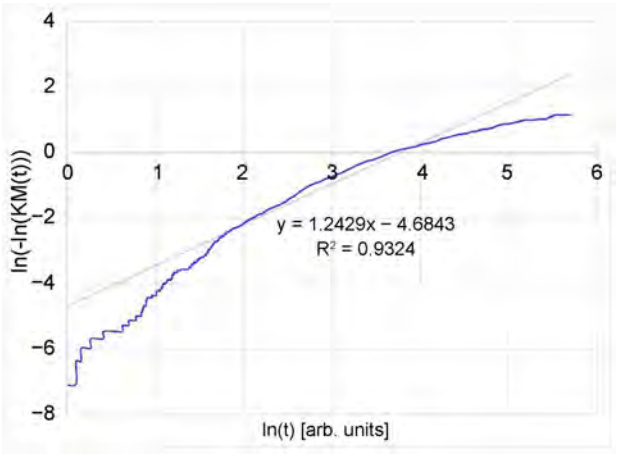


Figure 15. The logarithmic fits of WF to KM of Figure 10. The linear fit to the complete curve gives two parameters: $\mathrm{SE}=1.6544$; $r^2=0.9850$, $n=1.2429$; $t_0=43.33$; $S_{Sh}=4.664$.

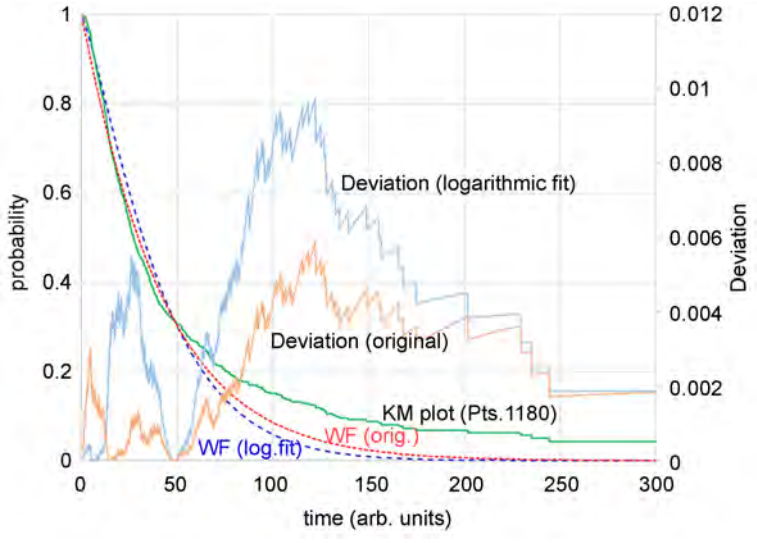


Figure 16. The logarithmic fits of WF to KM of Figure 10. The linear fit to the complete curve gives two parameters: $\mathrm{SE}=3.93$; $n=1.2429$; $t_0=43.33$; $S_{Sh}=4.664$. The deviation from the parameters of the original fit ($\mathrm{SE}=1.6544$; $r^2=0.9850$, $n=1.043$; $t_0=42.28$; $S_{Sh}=4.726$) shown in Figure 12(a).

Despite the inaccuracy of the logarithmic evaluation, it has a great advantage of guessing the subgroups of the patients by an optimal decomposing of the KM plot. The logarithmic curve on Figure 15. shows three well distinguishable parts, for which the linear is accurate, and divides the original KM into three subgroups, Figure 17.

The WF fit to Figure 17 of the three parts of the KM is shown on Figure 18.

The logarithmic fit by (22) shows different results than the direct fit. The reason is simply that the logarithmic fit considers only a part of the whole curve, and fits to that, consequently the accurate fit to that part of the KM will not fit to the other parts at all, if the logarithmic curve was approached in different parts. The observed KM is, of course, considers all the patients. The overlapping fits from the logarithmic approach modifies the KM plot. Consequently, only the fit for original KM plot has a relevance.

However, the logarithmic analysis is very useful for detecting the subgroups of the patients. It became clear that the survival contains three subgroups, Figure 17. Consequently, three partitions of the KM curve (Figure 10) would give a

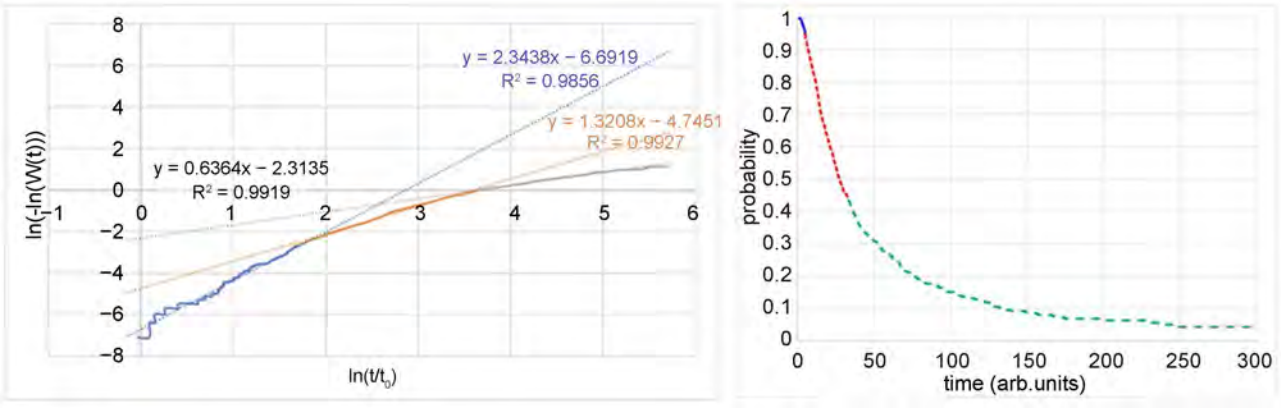


Figure 17. The various logarithmic fits to KM of Figure 10. (a) Linear fit to three fraction of the KM curve, $r_1^2 = 0.9856$, $r_2^2 = 0.9927$, $r_3^2 = 0.9919$, (b) Using the linear fits, the original curve may be fractioned to the three subgroups, (solid, dots and dashed lines).

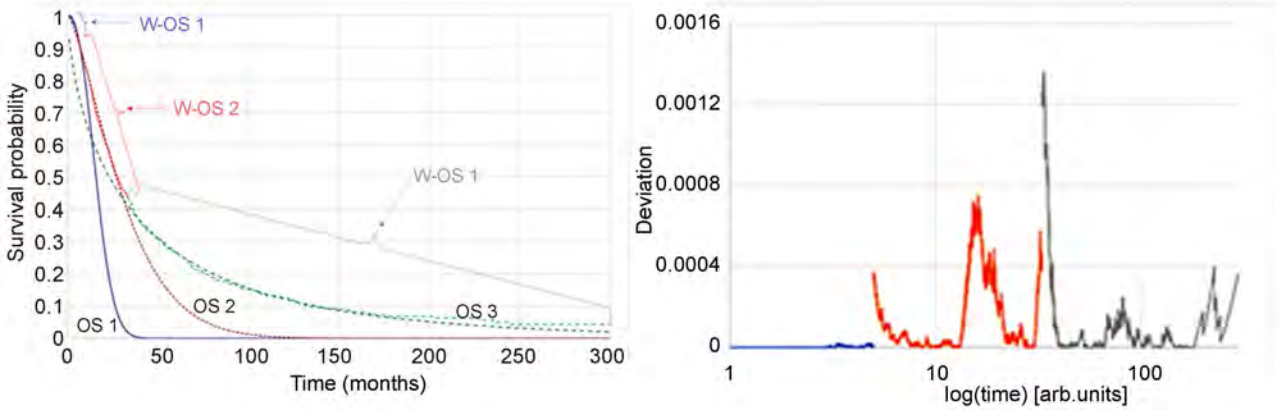


Figure 18. Fitting the KM by WFs according to the logarithmic fit on Figure 17. (a) curves, $n_1 = 2.344$, $t_{01} = 17.347$; $S_{Sh1} = 3.332$; $n_2 = 1.321$, $t_{02} = 36.328$; $S_{Sh2} = 4.454$; $n_3 = 0.6364$, $t_{03} = 37.913$; $S_{Sh3} = 4.758$. (b) deviations by groups.

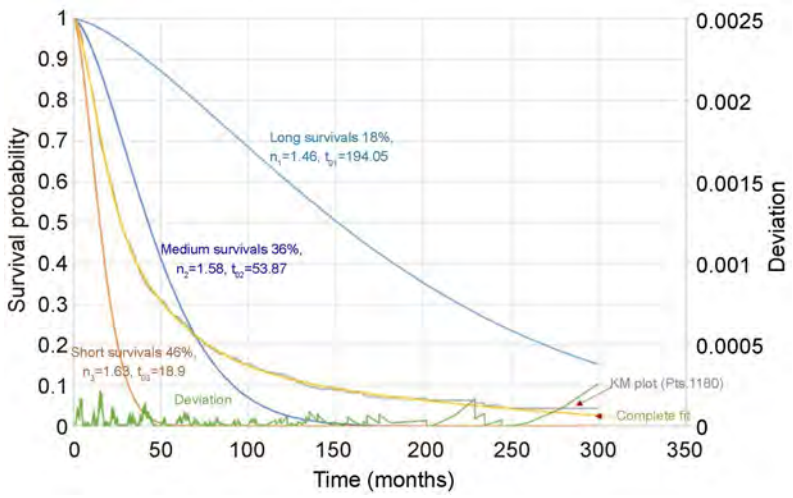


Figure 19. Decomposition of the KM on **Figure 10**. (Pts. = 1180) to three groups (long 18%: $n_1 = 1.46$, $n_1 = 1.46$, $\text{median}_1 = 151.02$, $S_{Sh1} = 6.071$; medium (36%): $n_2 = 1.58$, $t_{02} = 53.87$, $\text{median}_2 = 42.73$, $S_{Sh2} = 4.741$; short (46%): $n_3 = 1.63$, $t_{03} = 18.9$, $\text{median}_3 = 15.09$, $S_{Sh3} = 3.674$. The deviation of the fit remains under 0.0005 (0.05%).

more accurate fit, than the RG (**Figure 13**) or the two-group decomposition (**Figure 14**) had allowed. This fit to KM is very accurate, the deviation remains under 0.0005 in the complete fit, **Figure 19**.

5. Conclusions

We had shown the applicability of the two-parameter cumulative Weibull distribution for approximating the non-parametric Kaplan-Meier plot with a higher accuracy. We had shown the universality of the Weibull approach based on the general behaviors of the living organisms, including the cancer-tissue development. The self-organizing and self-similarity with their consequences determine the strict connection of the parametric approach well with the experimental non-parametric observations. Informational entropy allows the distinguishing of the subgroups in a general set of patients by their overall survival.

We have demonstrated that applying the two-parameter WF provides a sufficient fit to the non-parametric KM survival curve in a real case of 1180 patients suffering in various malignant diseases. Two of the 3 characteristic parameters of the KM plot (namely the points of median, mean or inflection) are enough to reconstruct the parametric fit.

In summary, Weibull parametric distribution with satisfactory refinement can accurately approximate a KM survival plot with surviving individuals at the endpoint of the study.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Parametrization of Survival Measures (Part II): Single Arm Studies

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Abstract

In some clinical applications in oncology randomized, double armed, and double-blind trials are not possible. In case of device applications, double-blinded conditions are nonrealistic, and with many times the randomization also has complications due to the high-line treatments where the reference cohort is not available; the active "arm" has mainly palliative initiative. Sometimes highly personalized therapies block the collection of the homogeneous group and limit its double-arm randomization. Our objective is to discuss the situations of the single arm evaluation and to give methods for the mining of information from this to increase the level of evidence of the measured dataset. The basic idea of the data-separation is the appropriate parameterization of the non-parametric Kaplan-Meier survival pattern by the poly-Weibull fit.

Keywords

Single-Arm Clinical Trial, Survival-Time, Decomposition of Survival Curves, Personalized Treatment, Observational Trial

1. Introduction

Survival studies most frequently use the Kaplan-Meier (*KM*) non-parametric estimate. The *KM* estimator is fixed by the duration of participation in the observation. Both the start of the observation time and the end of the observation of the individual by events (censored due to death or dropped out from the cohort) are not absolute and have inexplicit values. The precariousness flows from the differences between real lifetime to observational time. We summarize the characteristic points of the life of a cancer-patient in Figure 1. Periods out of observation

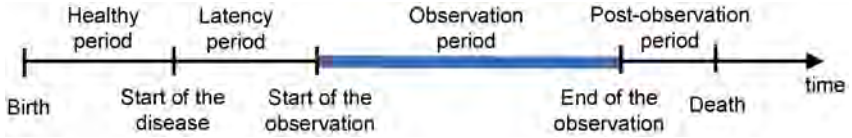


Figure 1. The time-scale of the individual participant's lifetime (Periods out of observation could be zero in any actual case, while the start of the observation could be a group of dates: the routine screening or the first symptoms and/or the first diagnosis).

could be zero in any actual case. One point (start of the disease) is elusive because the real symptoms of any disease could be later than the starting point of the disorder. This situation usually happens because the observation facilities of malignant diseases are technically limited. We could have only guessed the latency period, which starts with an avascular situation, forming a dormant microscopic cluster [1]. When the tumor leaves the dormant state by a scaling transformation [2], growth becomes traceable.

The real survival period in this content is blurry, so the definitions of the real survival points are the sum of the observation and the post-observation period. The evaluation may concentrate on disease-related death or any deaths in the observational period, irrespective of the cause. The observation period may only contain careful watch, then the treatment, and at the end, a long follow-up too. The observation period usually is evaluated statistically by the Kaplan-Meier non-parametric estimates (**Figure 2**). The end of observation could be decided by the endpoint of the study (e.g. 5 y survival), irrespective of the actual diagnosis of the patients at the end; or could be determined when all involved individuals have been censored or dead. In case of survival, the end could be determined when the patients of the studied group were cured and their state was declared NED (no evidence of disease). However, long (e.g. five years) survival does not necessarily mean a cured status [3]; a relapse of new metastases could happen in the post-observation period when in most of the cases new treatment starts.

The start of the observation could be after the routine screening when patients complain (about symptoms) and the statistically valuable period starts at the first diagnosis. The latent period can be long, even years before the discovery of cancer [4].

Measuring the effect of the treatment has various approaches, since having complications of the bio-variability and personal sensitivity of the treated individuals as well as the variation of the results depends on the social background and lifestyle of the patients. Randomized clinical trial (RCT) is a commonly used study design to measure lifetime. In an RCT, the active (investigated) arm can be statistically compared to the well-randomized control group in a carefully chosen, unified cohort. To evaluate a clinical intervention with the optimal possibility of RCT has ethical issues [5] [6], justification problems [7], and cohort-forming limitations. A crucial step of valid evaluation is, of course, selecting a group of patients who share common characteristics (cohort); otherwise, the variation of the results does not allow the estimation of the effect; discrepancies arise because of the patients' differences and not because of the therapy itself. Forming an

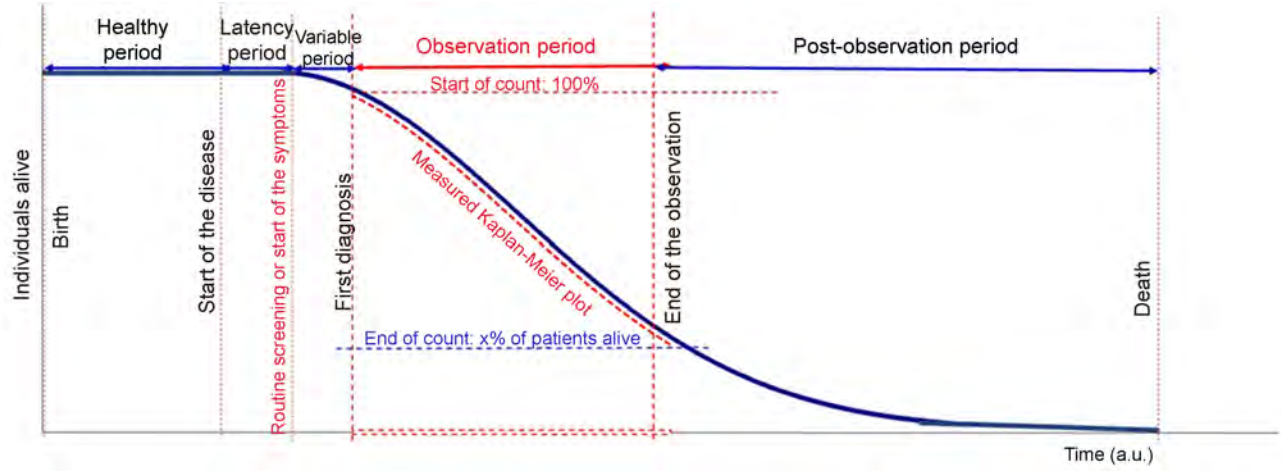


Figure 2. The time-scale of the group of participants' life-line (The lines for periods are naturally not definite; these are ranges of time-periods that could be overlapped too. The measured *KM* plot is the topic of interest for clinical trials).

appropriate cohort is a complex issue. Cohort forming sometimes uses forced conditions by reaching a definite toxicity predefined by the protocol (like in high-dose chemotherapy [8]), expecting the same (unified) reaction on the stage-selected patients. The RCT approach is devoted to the application of the most appropriate treatment update and for the reference control is used from the same cohort (called control-arm). The new therapy (active arm) must show its superiority over the control in comparison. The equipoise selection into both arms is mandatory, but the two treatments could be compared by not only their positive efficacy but their side effects as well, that may adversely affect the treatment [9].

Sometimes, in cancer treatment, a misleading (or at least not complete) evaluation is practiced by measuring the local control of the tumor, instead of the systemic development of the malignancy in the whole body. The problem of the overall control of the system is complicated and not even possible with imaging because of micro-metastases and such adverse effects which cause comorbidities for the patient. Therefore, parametrization would only be effective if the endpoint of the study is the overall survival and the quality of life combined.

Before deciding on the RCT, both sides of the balance of measured efficacy and the adverse effects must be taken into account. In case of serious diseases or terminal cases, no curative treatment is available, or further curative therapy is simply not possible because of comorbidities like organ-failure, low-blood-count, etc. Note that some conditions limit the RCT evaluation even in the double arm construction: the false inclusion and exclusion criteria (sometime "cherry picking"); the missing normal distributions; or the changing time series that have the same statistical momentums but their time-fluctuations differ. The data-set in the last case is out of the applicability of the usual analysis of variance (ANOVA). Furthermore, the ethical selection issues oppose the randomization, so the trial must be solved in a simple non-randomized design of single arm.

Due to the possible problems of RCT, some prospective clinical trials register

the data in the single arm only. The most frequent reason is the targeted far advanced disease where the conventional curative therapies have failed, and no other treatment is available except for the newly tried one. In these cases, the best supportive care (BSC) could be applied [10], like a control group when an active curative or palliative therapy is under investigation, and retrospectively, a historical control of the same hospital or large databases are also frequently compared to the historical data-set of the same hospital or compares to other large databases, retrospectively. There are some situations where no suitable historical control is available because of the completely new approach of the therapy [11], or the disease is so rare, that no comparison could be found [12]. Of course, we know that the single arm without a reference cannot give information about the changes that were achieved by the therapy involved. However, it is also obvious, that the data of the interesting changes are involved in the single arm spectrum as well but are well hidden without an orientation to measure the changes.

The single arm design is popular in the Phase I process when safety data is collected. The goal in this phase of the study is to determine the toxicity, the side effects and the dose with dose-escalation process. The investigation of efficacy is not included in Phase I trials. The Phase II studies concentrate on efficacy of the applied safe process [13]. When the hypothesis to be proved is clearly defined and the "null hypothesis" could be the zero response, the minimum of the clinically relevant response should define the size of the trial contrary to the simple design where the evaluation of the data can be rather complicated due to the difficulty of the missing reference for comparison, which is hard anyway because of the natural biological variability. The interpretation of the results of single arm distinguishes the placebo effect or the spontaneous natural history of the disease from the actual treatment efficacy. However, the single-arm trials may be the option when placebos are unethical, and opportunities of the controlled trial are limited, due to the vast variations of the patients. For example, the advanced diseases in oncology are frequent topics of single-arm trials, due to the massive, exhausting and mostly variant protocols of failed pretreatments. The reason of the failure is usually a progressive and refractory disease, or limitations in applying the conventionally proven methods due to organ-failure or a dangerous level of blood damages. In these cases, forming appropriate cohorts is very difficult or even not possible. When a single arm study is chosen due to the certain drawbacks of RCT, we mostly apply a palliative BSC additive to the active treatment. One of the most important condition of such single arm treatments is that it must not worsen the results of BSC, and its worst outcome must be the ineffectiveness. The best indicator of this condition is the combination of overall survival time and the quality of life.

2. Methods

Lifetime studies have a surprising universality by the self-organizing [14] [15]

and consequently by the self-similarity of the morphological structures and dynamic processes in living objects. Self-similarity has a morphological consequence, showing the spatiotemporal fractal structure in biological objects [16]; [17]. These ideas are forming the similarities of the species [18], which directly leads to the expected lifetime universality of well-selected cohorts. The general allometry is as wide as the cover of the mass, ranging from respiratory complexes, through the mitochondria, to the animals with the largest mass [19].

Due to the self-similarity, most of the biological structures and processes can be described by a simple-power function (like $P(x)=ax^{\alpha}$), where a and α are constants, and so the form of $P(x)$ remains only multiplicated by the constant during any m magnification of x: $P(mx)=a(mx)^{\alpha}=m^{\alpha}ax^{\alpha}=m^{\alpha}P(x)$. This magnification process (scaling [20]), could be followed by a few orders of magnitudes (scale-free behavior) in biosystems.

In consequence of the widely applicable universality behavior, the general ontogenic growth [21] allows the deduction of the Weibull distribution [22], which can be used to analytically describe the non-parametric Kaplan-Meier estimate for tumors. Self-similarity drives the tumor-development, which shows the universal law of growth [23] [24]. This lays the foundation of our attempt to find the reason behind the universal parametric regression for the lifetime of the patients, which is supported by the universal law of growth of the solid tumors [25]. The extension of the Weibull model allows us to estimate the tumor-latency too [26]. We had shown the self-similarity of bioprocesses in general [27], leading us to some well-defined mathematical formulas like the Avrami equation, which has a complete formal correspondence with the function of the cumulative Weibull distribution (WF) [28]. The two-parameter cumulative Weibull distribution (WF) is a good candidate for the parametrization of the *KM*-plot [27]. It is both theoretically and practically established for clinical applications [29].

The real challenge is how we can reveal the hidden data in the single active arm in case of the missing randomization that forms reference in double arms. We have limited possibilities for mining the available information without a reference set, even though we know it well, that the information is in the data. The general self-similar behavior of the various tumors has different parametrization and so can be distinguished from each other. Consequently, the fitting to survival curves gives hints on how to extract information from the single arm alone.

Experimental data fit well to the empirical data in biology as well as it has been widely investigated and proven in solid-state reactions (precipitations, phase-transitions, aggregations, nucleation, growth, and others) [30] [31] [32] [33] [34]. Indeed, experimental data show that many biological reactions follow the Avrami equation. It is applied universally to different processes regardless of the structure and dynamics of the system. Avrami functions are self-similar, and various comparative functions characterize the exponents [35]. The considerations of Avrami function explain the parametric approximations of the non-parametric Kaplan-Meier survival distribution (*KM*) [27].

The mortality can be approached by the fitting of different distributions [36]

in epidemiologic modeling. The most popular descriptions are the Gompertz, Weibull and logistic distributions [37]. These methods are usually used for gerontologic, aging mortalities, modelling the statistics of the ages of death, do not consider any particular disease or clinical therapy involvements [38] [39] [40] [41]. A generalized Weibull-Gompertz distribution could derive various distributions [42]. In demographic aging, the Gompertz and Weibull functions describe different biological causes [39]. The Gompertz model involves a multiplicative aging mortality, while it is additive in Weibull description. The multiplicativity affects the extrinsic, while the additivity the intrinsic causes in older ages. Our present modeling does not deal with aging mortality and the connected epidemiologic consequences. Our considerations comprise the cancer-survival, which is strongly disease and therapy dependent, so it covers the intrinsic causes, on the actual parametrization of the probability of survival. This non-aging survival discussion prefers the Weibull distribution in comparison to Gompertz, describing the intrinsic self-organiziation behaviour of the human living organism.

In such advanced situations, when the malignancy is double refractory, the WF provides the best fit to the *KM* [11]. The cancer incidences significantly fit Weibull distribution in 18 types of malignancies [43], and so WF is justified to describe the driver events of the tumor-building process. Extending this idea, we expect that the best fit parametrization of the survival curve could lead to the information about the hidden facts in the actual non-parametric *KM* plot.

The approximation with a simple WF function in real cases of the *KM* non-parametric survival curve is not precise enough. The missing preciosity apparently contradicts the WF self-organized basis. When the survival is self-organized in the same way as we observed in all the biological processes, the fitting to the non-parametric *KM* has to show the self-similarity, because it is entirely rigorous due to the universality of the lifetime of the living systems and the growth dynamics of the tumors. The contradiction is due to the fact that the self-similar WF only fits to strictly homogeneous patients' cohorts. WF parameters characterize the group of generally equal participating individuals, which is of course not acceptable. The *KM* represents a cohort group of patients with the equipoise of individuals made as ideal as possible, choosing explicit inclusion and exclusion criteria. Nevertheless, the choosing criteria in the situation when we are not able to apply RCT cannot be fixed well. The only inclusion is the failure of conventional curative treatments and the only exclusion is when the patient is in such terminal stage when any extra intervention could be fatal.

Due to the enormous variability of the living conditions (like social, diet, habits, etc.) and bio-variability of the individuals (like genetic variability, immune-variability, sensing-variability, etc.), any chosen cohort has inhomogeneities. However, it is possible to divide the cohort into more homogeneous subgroups than the full set of individuals, expecting that the fitting of the self-similar WF will be better by the growing homogeneity of the subgroup to which it is applied.

Usually, the groups of local responses (complete response (*CR*), partial response (*PR*), no change (*NC*), or progression of the disease (*PD*)) come into the center of the attention automatically at the finishing of the study. We could make similar subgrouping in systemic (lifetime, survival) measurements, and WF fit them individually. The measured data is the summary of the complete cohort with overlapping data in the experimental non-parametric *KM* estimates, containing the data of all the subgroups. For simplicity, using the same subgrouping as in local response, the subgroup of those patients who could be regarded is introduced as "cured" (*CP*), the subgroup for those whom the treatments helped (they as responding patients (*RP*), and the patients who had no benefit from the therapy as non-responding patients (*NP*). The *KM* in the real experiment measures is only the sum of these (in the same way as in the analysis of the local response). Fit WF for subgroups and sum it for fitting to complete *KM*:

$$W^{(KM)}(t)=\frac{n_{CP}}{N}\mathrm{e}^{-\left(\frac{t}{t_0^{(CP)}}\right)^{n^{(CP)}}}+\frac{n_{RP}}{N}\mathrm{e}^{-\left(\frac{t}{t_0^{(RP)}}\right)^{n^{(RP)}}}+\frac{n_{NP}}{N}\mathrm{e}^{-\left(\frac{t}{t_0^{(NP)}}\right)^{n^{(NP)}}} \quad (1)$$

$$\text{and}\quad n_{CP}+n_{RP}+n_{NP}=N$$

where n_{CP}, n_{RP}, n_{NP} are the number of patients in *CP*, *RP* and *NP* groups, and *N* is the number of patients in the complete cohort. Note, that the difference between the *CP* and *RP* groups is only in the definition, just like in the local response between the *CR* and *PR* categories. Usually *CP* can be defined to the lifetime of the healthy group of patients in an age-normalized comparison. Consequently, for easy categorizing, usually the *CP* is the long, *RP* is the medium and *NP* is the short survival.

Simpler and more roboust WF regression received, when the fitting is divided into only two different functions [44]. Here we define two sub-cohorts composed linearly [45] [46] [47], one that the treatment had no or minor influence on (*NP*) and one where the treatment was effective (*RP*):

$$W^{(KM)}(t)=c_{RP}\mathrm{e}^{-\left(\frac{t}{t_0^{(RP)}}\right)^{n^{(RP)}}}+c_{NP}\mathrm{e}^{-\left(\frac{t}{t_0^{(NP)}}\right)^{n^{(NP)}}} \quad (2)$$

where the Weibull parameters denoted by (*RP*) and (*NP*) superscripts, according to their sub-cohorts. Due to the complete set of patients, $c_{RP}+c_{NP}=1$, so (2) is:

$$W^{(KM)}(t)=c_{RP}\mathrm{e}^{-\left(\frac{t}{t_0^{(RP)}}\right)^{n^{(RP)}}}+(1-c_{RP})\mathrm{e}^{-\left(\frac{t}{t_0^{(NP)}}\right)^{n^{(NP)}}} \quad (3)$$

Using the regression with division into only two subgroups by temperature development criteria was used by others [48] where the patients included in the hyperthermia cohort were divided into "heatable" and "non-heatable" sub-groups, where the end of the study was determined by the time when the last patient was proved to be unaffected by hyperthermia. Two (responding and non-responding) or more subgroups (including the stabilization, treating a chronic disease, or other), could be introduced this way as well.

The two-subgroup division has five parameters to fit. Looking for the only

concentration parameter ($c = c_{RP}$), some examples look like it is shown in Figure 3.

In that special case when the *RP* subgroup is cured, meaning no disease-specific death happen in the whole observation period (including the available follow-up time too), the $e^{-\left(\frac{t}{t_0^{(RP)}}\right)^{n^{(RP)}}} \cong 1$, so the WF-like curve will have the following form:

$$W^{(KM)}(t) = c_{cure} + (1 - c_{cure}) e^{-\left(\frac{t}{t_0}\right)^n} \tag{4}$$

According to our general knowledge in oncology, the size of the malignant tumor certainly affects the lifespan of the cancerous individuals. The ratio of the actual basal metabolic rate (basal energy consumption) of the malignant lesion $E(t)$ to the healthy one E_0 with the same volume modifies the survival distribution ($P_S(t)$) which modifies the simple Weibull-related distribution as follows [24]:

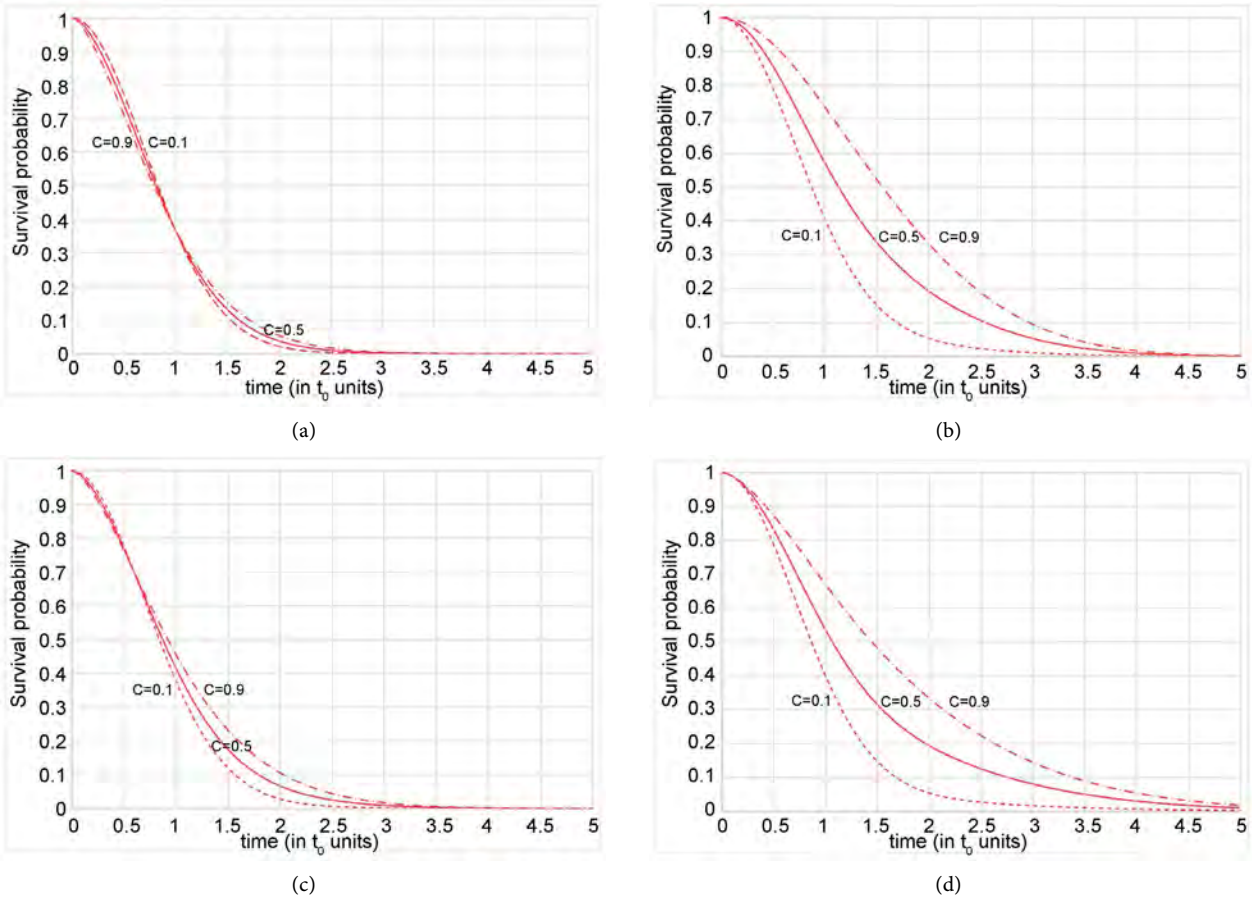


Figure 3. Examples of the fitting curves at various c-values, where (a) equal the time-factor: $n^{(NP)} = 2$, $t_0^{(NP)} = 1$, $n^{(RP)} = 1.5$, $t_0^{(RP)} = 1$; (b) equal the shape-factor: $n^{(NP)} = 2$, $t_0^{(NP)} = 1$, $n^{(RP)} = 2$, $t_0^{(RP)} = 2$; (c) changing by a 20% increase of the time-factor in real mix $n^{(NP)} = 2$, $t_0^{(NP)} = 1$, $n^{(RP)} = 1.5$, $t_0^{(RP)} = 1.2$; (d) changing 100% increase of time-factor: $n^{(NP)} = 2$, $t_0^{(NP)} = 1$, $n^{(RP)} = 1.5$, $t_0^{(RP)} = 2$.

$$W_S(t) = \exp\left(-\frac{E(t)}{E_0}\left(\frac{t}{t_0}\right)^n\right). \quad (5)$$

The modification of (5) can be interpreted as the change of the t_0, and the scale factor of the Weibull function:

$$t_0' = t_0\left(\frac{E(t)}{E_0}\right)^{-1/n} \Rightarrow W'(t) = \exp\left(-\left(\frac{t}{t_0'}\right)^n\right) \quad (6)$$

Consequently, the scale-factor of WF (the time-factor of survival fit) contains the information about the tumor-growth in the way it was shown in (6). The original Weibull-based parametric approach of *KM* survival curve from the 0th stage gives a reference to the E_0 value.

On this basis we study the changes of the two Weibull-parameters by fitting the cumulative distribution curve to the hypothetical choice of the survival studies in different stages of the disease, which is directly connected to the inclusion criteria of the study. Also, we follow the change of parameters by the endpoint of the studies fitting to the finishing conditions. The mathematical fit of the curves uses the least square method by digital stepping of the functions in large number (n > 1000) steps and optimizing the square of Pearson parameter (maximize) and also the sum of squares of deviations (minimize). We used two software supports: the Excel (Microsoft 365) and the MathCad 15.

3. Results

Using the hypothesis, that the self-similar WF follows the real bioprocesses in survival, the effect of the malignancy staging at the first diagnosis could be followed with the Weibull fitting method, hypothesizing, that the staging strongly correlates with the time of the first actual diagnosis in the same cohort of patients. Diseases discovered earlier have lower stages than the ones diagnosed later. First, we are dealing with the survival curves of the patients in the control arm (reference arm, which in principle could be placebo as well), so the treatment modification will be considered later.

The start of the treatment is not immediate. Even the most accurate and modern detection methods do not allow the diagnosis in a latent state. The earliest time when the first diagnosis can be made is only after the dormant (untraceable) period of the disease. The traces of the disease cannot be detectable by imaging (due to its lower sensitivity), but some blood-test could detect the signal of disseminated circulation cancer cells or its parts. Overall Stage Grouping uses stages 0, I, II, III, and IV to characterize the progression of cancer [49]. Stage 0: when the cancerous cells are observed very locally without an observation anywhere else (carcinoma in situ); Stage I: cancers are well localized; Stage II: cancers are locally advanced and affect the sentinel lymph node or nodes only in one side of the tumor; Stage III: cancers are regionally advanced, the affected lymph-nodes are around the tumor; Stage IV: cancers have distant metastases. WF function could extrapolate the undetectable period from the fittings to the

actual clinical stage of the tumor [25]. The extrapolation of Weibull regression considers the time when the study starts, which is of course later (earliest detectable stage after dormancy) than the start of the tumor-process. The space-resolution of the most frequent imaging methods in clinical practice resolves the tumor in a 10^{-2} m range, which is about 1 cm^3 volume, having already billions of tumor-cells. Supposing a cluster contains 30 cells (~3 cells in a diameter) and supposing it takes 100 days to double its size, the tumor will be in the preclinical (latent) state for approx. 8 years, without the existing malignant tumor being observable, but we assume the self-organized growth during this time-period too.

Considering the basic survival curve from the start of the malignant behavior even from a single "renegade cell" [50], the WF describes the tumor development including the dormant period until all the patients deceased or censored, (we obtain (7):

$$W_b(t) = \mathrm{e}^{-\left(\frac{t}{t_0^{(b)}}\right)^{n_b}} \tag{7}$$

Following the staging of the tumor status with WF when the diagnosis is based on the development of the malignant lesion related to (5):

$$W_S^{(i)}(t) = \exp\left(-\frac{E_i(t)}{E_0}\left(\frac{t}{t_0^{(i)}}\right)^{n_i}\right) \quad (i = \mathrm{I, II, III, IV\ stages}) \tag{8}$$

Hence, according to (6), the measured $t_0^{(i)}$ in subsequent stages from

$$t_0'^{(i)} = t_0\left(\frac{E_i(t)}{E_0}\right)^{-1/n_i} \Rightarrow W_i(t) = \exp\left(-\left(\frac{t}{t_0'^{(i)}}\right)^{n_i}\right) \quad (i = \mathrm{I, II, III, IV\ stages}) \tag{9}$$

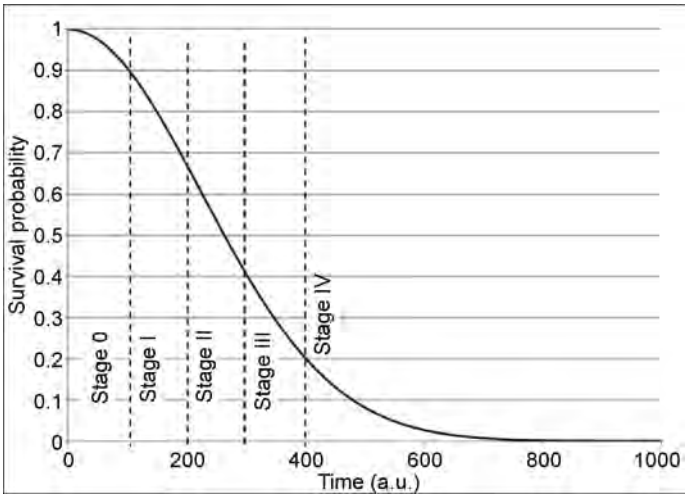
Let us denote the time when the tumor is observed like in carcinoma in situ, by T_0. Due to the supposed continuity of the tumor-growth from the latent to the observable stage, the WF fit could follow triple parametrization to the *KM* non-parametric estimate. In this case a location parameter is added to the shape and scale parameters:

$$W_0(t) = \mathrm{e}^{-\left(\frac{t+T_0}{t_0^{(0)}}\right)^{n_0}} \tag{10}$$

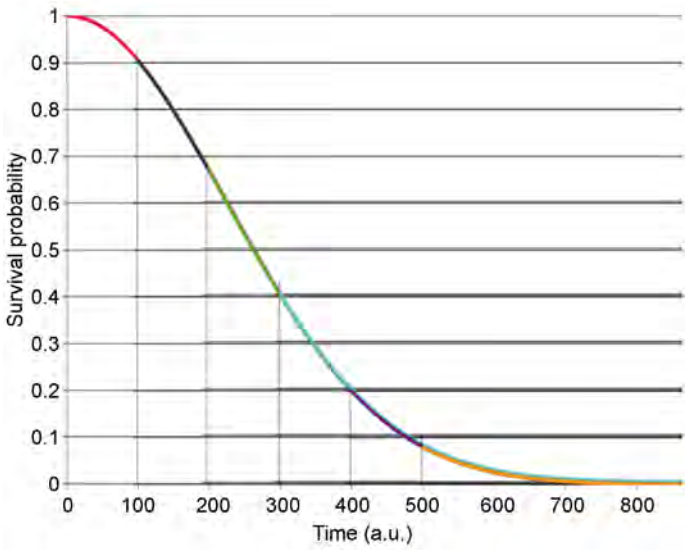
This gives a "truncation" possibility of this basic (Equation (7), hypothetical) overall survival plot (**Figure 4**).

Following the complete survival until the last event (or censoring) in the studied group of patients, the start of the study will be at the shifted time, which determines the truncations of the basic WF to its parts (**Figure 5**).

The survival studies of different stages could be regarded as studies in shifted time (T_i), starting the observation of the patients (first diagnosis) a certain time later than the guessed start (stage 0) of the malignant process. The new start is of course regarded as a new study, considering again 100% of the patients who are involved in this stage, with a probability of 1. The truncated curves (**Figure 5**) considered as the new studies, that could be WF fitted with modified parameters.



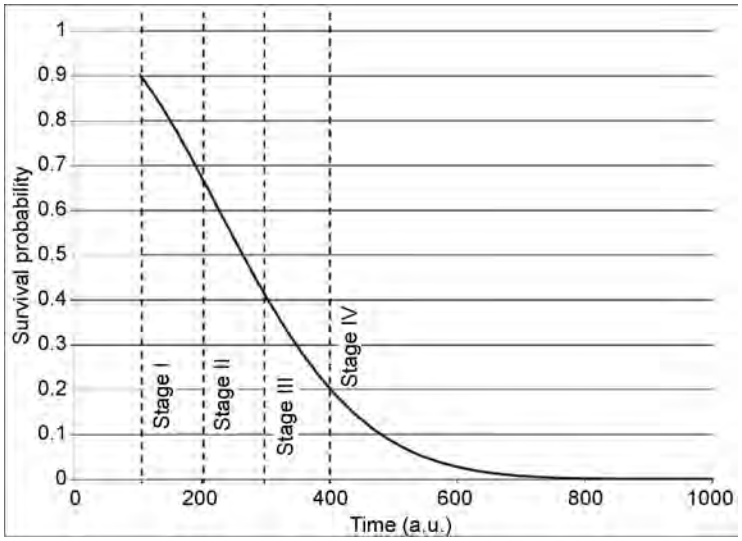
(a)



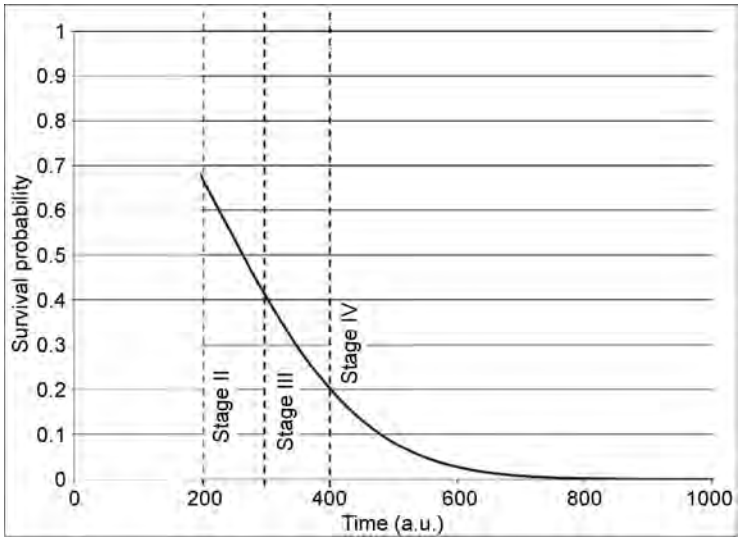
(b)

Figure 4. A hypothetical stage grouping of overall survival. 0: carcinoma in situ; I: well-localized lesions; II: locally advanced, affected the sentinel lymph-node; Stage III: regionally advanced, affected lymph-nodes; Stage IV: distant metastases. Parameters of the original WF are $n = 2$, $t_0 = 316$. (a) cut by stages, (b) various parts are colored.

Screening could be misleading for survival evaluations because sometimes the elongation of overall survival with a certain time is an addition to the differences between the first diagnosis [51] and the overall survival. We expect that the earlier discovery of the tumor extends the survival by more than the time difference between the first diagnosis and the discovery of the symptoms. Consequently, a certain change of the scale factor (t_0) does not consider any treatment in the truncated periods due to the obvious shortening of the survival when we truncate the constant WF function. Of course, despite the unchanging type of the tumor, there is no guarantee for the constant shape-factor of survival in various stages. The change of the tumor-size changes the micro- and macroenvironment


Survival probability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
Time (a.u.)
Stage I
Stage II
Stage III
Stage IV

(a)


Survival probability
1
0.9
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0.4
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400
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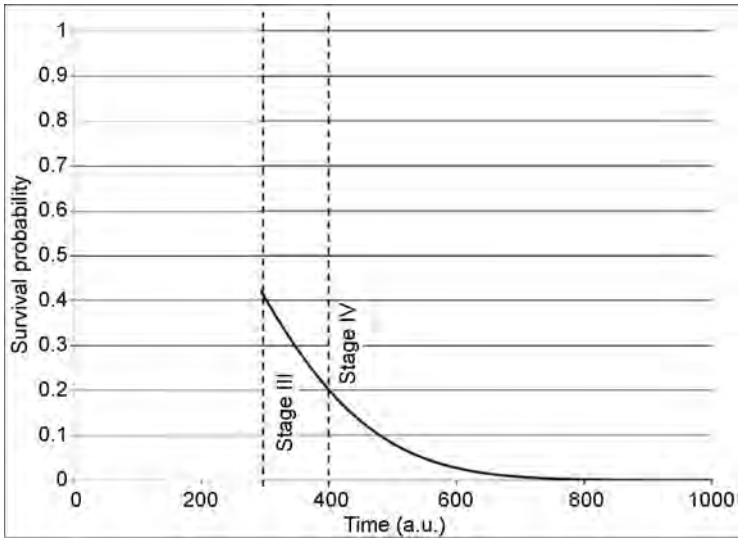
1000
Time (a.u.)
Stage II
Stage III
Stage IV

(b)


Survival probability
1
0.9
0.8
0.7
0.6
0.5
0.4
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200
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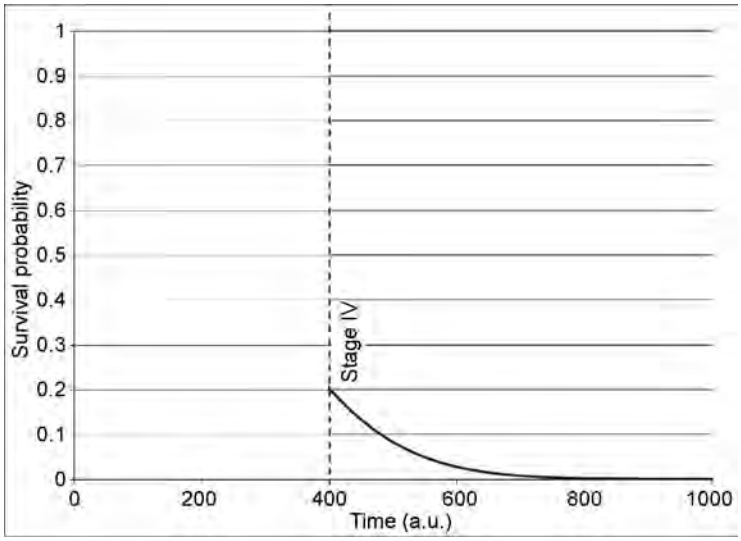
800
1000
Time (a.u.)
Stage III
Stage IV

(c)



(d)

Figure 5. The remaining parts of the original (basic) WF truncated accordingly to the subsequent stages. (a) The tumor is diagnosed in stage I; (b) The tumor is diagnosed in stage II; (c) The tumor is diagnosed in stage III; (d) The tumor is diagnosed in stage IV.

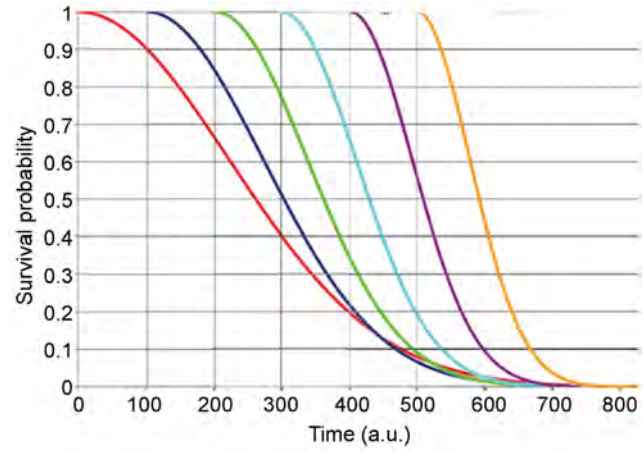
of the tumor, reorganizes the complete structure in the lesion, so the shape parameter also changes. Note, that normally different tumors can be detected in different stages. For example, most of the breast and cervical cancers are detected in the stages 0 or I, while lung cancer is usually detected in stage III or IV, depending on the observed symptoms or the accident screening without indicated complaints of the patient. Due to the developing technical conditions, the complete process depends on the historical time of the screening.

Considering T_i, the shift for the studies in subsequent stages, we get:

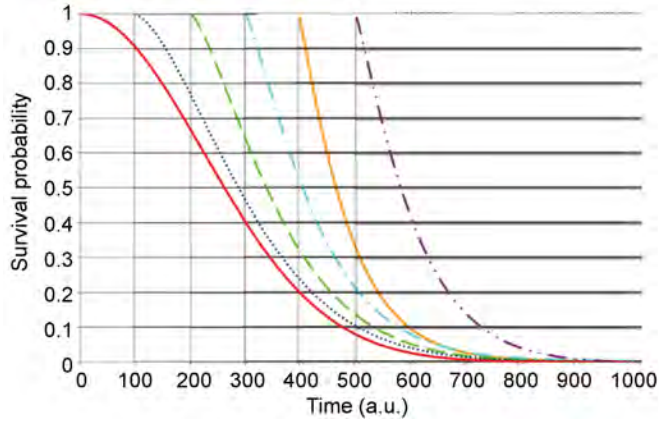
$$W_i(t) = \mathrm{e}^{-\left(\frac{t+T_i}{t_0^{(i)}}\right)^{n_i}} \tag{11}$$

The T_0 is the start of the observational period: optimally the immediate treatment, or at least the watchful waiting (watch and wait, WAW period); when the treatment cannot be decided yet. For simplicity we consider the studies as time-to-event (TTE) data, where time is denoted from a starting point to a certain event, such as death. When the end of the study fixed differently, we must use the fit shown in (2). All studies start as new one, of course, there is no knowledge about the unmeasured early treatments; consequently, survival probability at the start of the treatment is 1, irrespective of when it started. We show the later starting points in the time-line of the disease in **Figure 6**.

We start counting the elapsing time from T_i, by time-shift in (12). The complete time-scale is shifted by T_i value. The number of patients at the starting of the trial is considered 100% for *KM*, consequently, the truncated "remains" must be normalized to 1 to be able to fit with WF fitted. Usually the cancer in T_0 does not cause symptoms for the patients. When the symptoms appear, and a



(a)

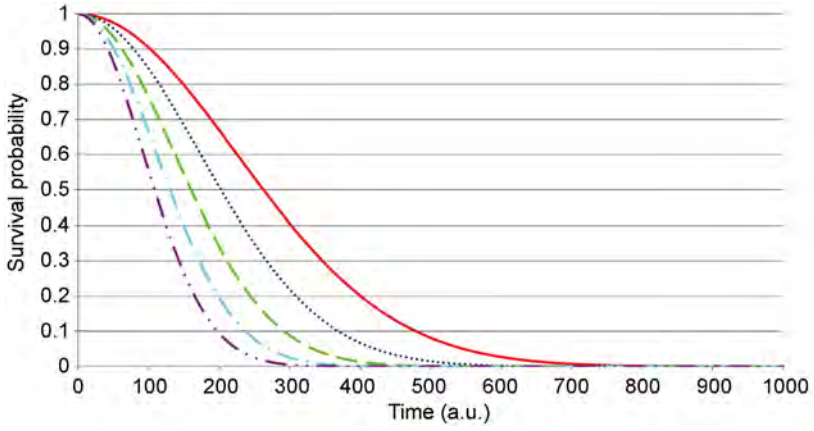


(b)

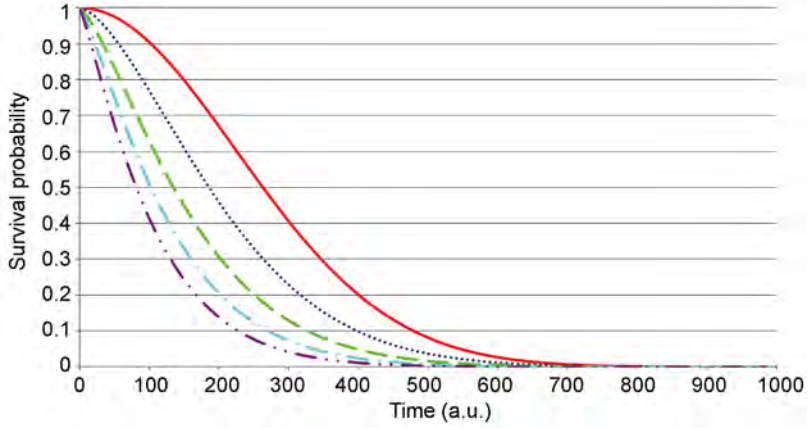
Figure 6. Late starts and WF-fits to the truncated curves that are shown in **Figure 5**. The original WF parameters: $n_b = 2$, $t_0^{(b)} = 316$, (solid line). (a) Curves have the same shape parameter as the original, but the treatment was started in one of the subsequent T_i time; $T_0 = 0$, $T_1 = 100$, $T_2 = 200$, $T_3 = 300$, $T_4 = 400$, $T_5 = 500$, the shape parameter is a fixed constant as the characteristic value of the actual disease. (b) is the same as (a), but WF is optimally fitted to the new conditions, therefore the shape parameter decreases.

patient recognizes the problem, it is usually in a later stage, when a higher number of cancer cells are already present, or even when they have already been disseminated from the local site. The WF fittings to the truncated "remains" (not showing the carcinoma in situ 0th stage), are shown in **Figure 7**. Calculation of the shape scale factors was made when the shape kept being constant (meaning the disease is the same in all the studies, irrespective of its starting time). Another calculation showed an optimal Weibull fit, when both the scale and shape factors changed. The idea is that in spite of the same disease, the late start met different conditions of the disease from the in-time beginning.

The curves in **Figure 7** could be considered as the start of the treatment in various stages (or TNM state) of the disease. The n_i and $t_0^{(i)}$ parameters have



(a)



(b)

Figure 7. WF fits of late starts on truncations which are shown in **Figure 5**. The original WF parameters: $n_b = 2$, $t_0^{(b)} = 316$, (solid line). (a) Curves have the same shape parameter as the original. Other parameters are: $T_1 = 100$, $t_0^{(1)} = 234.6$, (dotted line); $T_2 = 200$, $t_0^{(2)} = 192.8$, (dashed line); $T_3 = 300$, $t_0^{(3)} = 156.6$, (dashed-dotted line); $T_4 = 400$, $t_0^{(4)} = 130.5$ (dashed-double-dotted line); (b) Curves are modified by shape for best fit. The parameters: $T_1 = 100$, $n_1 = 1.57$, $t_0^{(1)} = 234.3$, (dotted line); $T_2 = 200$, $n_2 = 1.35$, $t_0^{(2)} = 176.7$, (dashed line); $T_3 = 300$, $n_3 = 1.23$, $t_0^{(3)} = 137.9$ (dashed-dotted line); $T_4 = 400$, $n_4 = 1.16$, $t_0^{(4)} = 111.3$ (dashed-double-dotted line).

logarithmic dependence on the T_i late start time in **Figure 8**.

In reality, the real *KM* curve could be decomposed to at least two components like it is shown in (2). An example is shown in **Figure 9**, where the disease is characterized by the same shape factor, only the scale factor changes from 1 y (non-responding) to 10 y (responding) situations. When the later start of the study is linearly changed we assume linearity of the decomposition factor too.

The form of **Figure 9** shows the general figures of the comparison of studies started in different stages of the same malignant disease well.

The late (at a more serious stage) start of the treatment is not the only challenge in the evaluation. Another common challenge at the *KM* evaluation is the

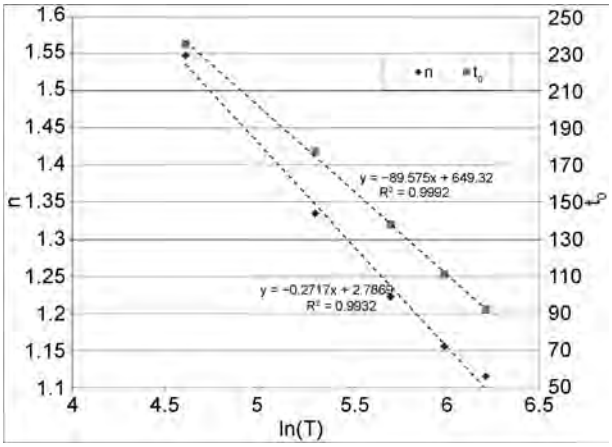


Figure 8. Fits n_i and $t_0^{(i)}$ vs. $\ln(T_i)$ when the original WF ($n_b = 2$, $t_0^{(b)} = 316$) had been truncated [$n(T) = -0.27\ln(T) + 2.79$, ($r^2 = 0.993$) and $t_0(T) = -89.58\ln(T) + 649.3$, ($r^2 = 0.999$)] (data are from **Figure 7(b)**).

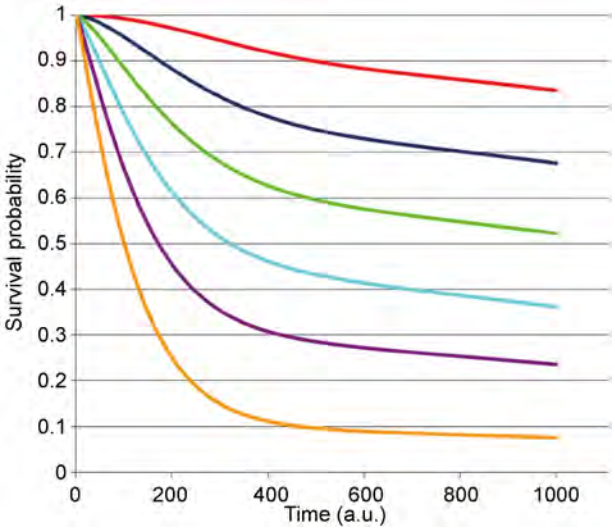


Figure 9. The *KM* curves for different stages (the study started at different times), where the *KM* is decomposed from two WFs. Original WFs for responding and non-responding patients have: $t_0^{(RP)} = 3650(10\text{ y})$, $t_0^{(NP)} = 365(1\text{ y})$; $n^{(RP)} = n^{(NP)} = 2$. The actual decomposition factors from up to down are $c_0^{(RP)} = 0.9$, $c_1^{(RP)} = 0.75$, $c_2^{(RP)} = 0.6$, $c_3^{(RP)} = 0.45$, $c_4^{(RP)} = 0.3$, $c_5^{(RP)} = 0.1$ to the late-start times $T_0 = 0$, $T_1 = 100$, $T_2 = 200$, $T_3 = 300$, $T_4 = 400$, $T_5 = 500$, respectively.

end-time of the study. Most of the clinical studies have limited time for follow-up, so they are usually finished before all involved patients are deceased or censored, and they do not force the TTE condition. At the end of the study, a certain group of patients remains (patients at further risk, *PFR*), or patients are completely cured (PCC). Identifying the PCC group in the practical applications is very unprecise, and by definition, the *PFR* at five years point regarded as PCC. However, there are doubts about this strict limit [3], so we use the *PFR* only, without declaring the PCC. The end-time-point of the study is the preplanned

goal, and the patients in the *PFR* group are censored at this point. This time-limit causes a certain early truncation of the hypothetical overall-survival curve. The hypothetical curve fit to *KM* is WF when the study goal is TTE; so it would be continued to the complete end (all patients deceased or censored, no patients are at risk). The finish-times (F_i) define the *PFRs* in actual points, when N patients were involved in the study:

$$\frac{PFR_i}{N} = \exp\left(-\left(\frac{F_i}{t_0}\right)^n\right) \tag{12}$$

where the PFR_i values are patients that are alive (they are at risk, belonging to the actual *PFR*) at the early finish time when the actual study ends. When the study finishes before all events happen at F_i, the patients at risk is PFR_i, and the number of events (loss of patients due to death or censored) until this point will be: ($N - PFR_i$). The finish of the study (F_{last}) is when a single patient remains at risk ($PFR = 1$), and censored from the initial set of N individuals,

$$F_{last} = t_0\left(-\ln\left(\frac{1}{N}\right)\right)^{\frac{1}{n}} \tag{13}$$

According to the Hardin-Jones-Pauling's (HJP) biostatistical theory [52] [53], we expect the death of the last patient by the time of the average survival of the actual study is after the trial is closed. Consequently, the hypothetical complete length of the study would be

$$F_{end} = t_0\left[\left(-\ln\left(\frac{1}{N}\right)\right)^{\frac{1}{n}} + \Gamma\left(1+\frac{1}{n}\right)\right] \tag{14}$$

The early finished studies, when a certain number of patients remain in risk are shown by an example in **Figure 10**.

The studies finishing early have a slight shift in t_0 when elongating them and the number of patients at risk decrease (**Figure 11**).

4. Discussion

Both the two independent Weibull parameters change by inclusion criterial of staging. Both the shape and the scale factors are decreased when treatment starts later, which is natural. In case of an unchanged *n* shape-character, the decrease of the scale factor is less than in case of a changing *n*.

Using (9) we get:

$$t_0^{(i)} = t_0\left(\frac{E_i(t)}{E_0}\right)^{-1/n_i} \Rightarrow E_i(t) = E_0\left(\frac{t_0^{(i)}}{t_0}\right)^{-n_i} \quad (i = \mathrm{I, II, III, IV\ stages}) \tag{15}$$

Expression (16) allows an approximating of the metabolic rate from the change of $t_0^{(i)}$ by WF fit to various *KM* non-parametric estimates. Metabolic activity could be measured approximately by positron emission tomography (PET), evaluating the standardized uptake value (*SUV*) of the radiolabeled tracer

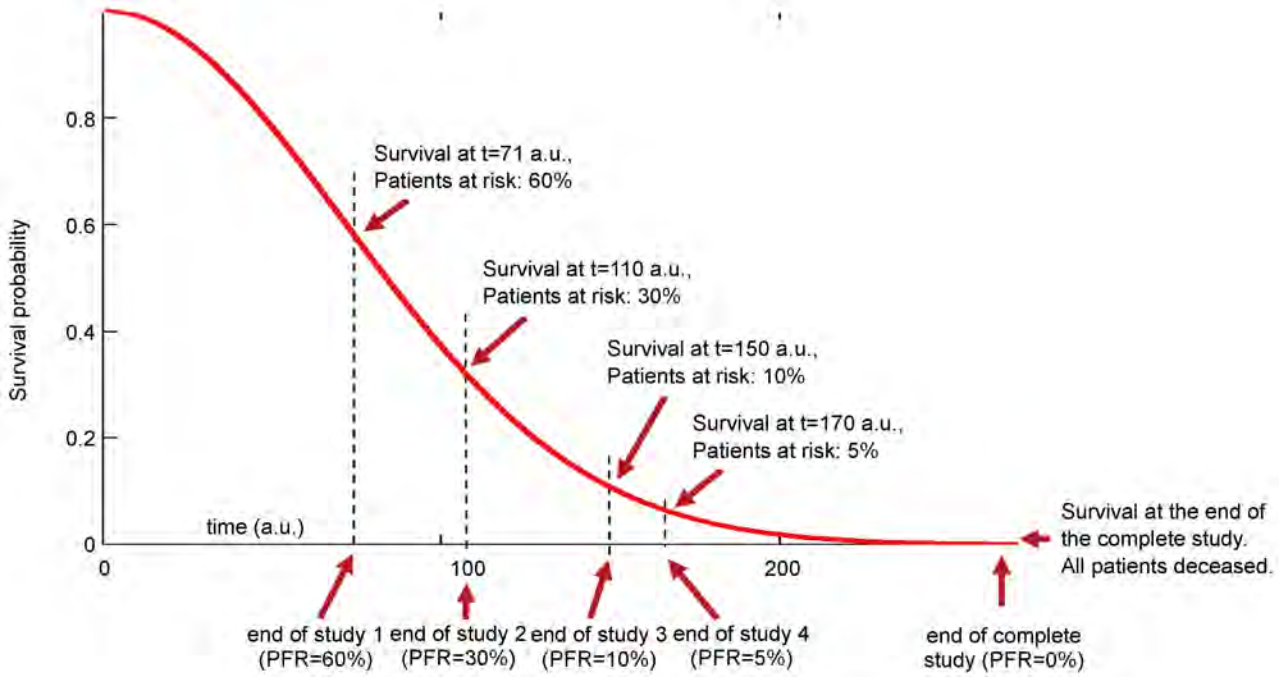


Figure 10. The hypothetical variation of the finishing of a study when a certain number of patients remain at risk (The parameters of basic WF are $n=2$; $t_0=100$, $N=100$).

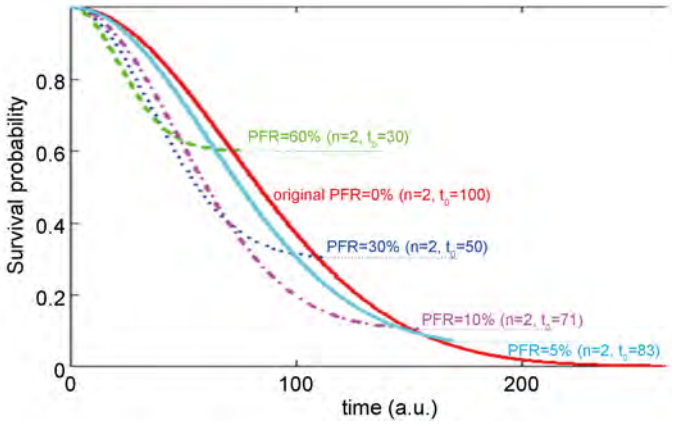


Figure 11. The different studies finished before all events happen (PFR = 5, 10, 30, 60 percentages). Note the changes of the t_0 value. The parameters are 83, 71, 50, 30 time units, respectively. The parameters of the complete KM are $n=2$; $t_0=100$, $N=100$.

2-deoxy-2-[18F] fluoro-D-glucose (FDG) uptake in tumors in various stages at the start of the trial (SUV_i), so:

$$\left(\frac{E_i(t)}{E_0}\right)=\left(\frac{t_0^{(i)}}{t_0}\right)^{-n_i} \Rightarrow \left(\frac{SUV_i(t)}{SUV_0}\right)\approx\left(\frac{t_0^{(i)}}{t_0}\right)^{-n_i} \quad \left(i=\mathrm{I,II,III,IV\ stages}\right) \tag{16}$$

where SUV_0 is the FDG uptake of the neighboring healthy tissue. The metabolic ratio, calculated by $\left(\frac{t_0^{(i)}}{t_0}\right)^{n_i}$ at the late start process above gives a quite

accurate linear dependence from the T_i late start time (Figure 12).

In this way we could also approximate the basic survival curve, when the PET is actually sensitive enough to measure cancer in situ lesions, supposing the time when the tumor starts to form in a microscopical region and its clusters are still undetectable with our present diagnostic methods.

The treatment of the chosen patient cohort is expected to change the *KM* of the active arm compared to the control arm, which is untreated with the same protocol, and formed from the same cohort. The changes of *KM* in active arm will modify the WF fit, too. The measured change of metabolic rate by *SUV* indicates the effect of the actual treatment. When the malignant tissue shows a lower metabolic rate (lower *SUV* ratio) the treatment regarded effective. The lower *SUV* has a longer scale parameter (t_0) according to (17). In case of a successful treatment, the shape-parameter (n) decreases, "smooths" the probability of event with a longer, heavier tail.

The question is: how the situation changes by treatments in the study? The WF changes of course and the evaluation use this change to compare it to the reference (control arm) WF. There are different parametric estimations for the result. The first attempt is always the median survival, which looks undecided about the efficacy of the treatment in the measuring process. However, this single parameter is not nearly enough to see the complete picture. It is possible that the treatment is effective without the change of the median of the *KM*, while the distribution has a long tail; patients over the median lifetime live longer. for example Figure 13. It can happen when the mortality of the disease is very rapid, and the development of the resistance made by the treatment needs a longer time compared to the median survival.

For the decision of the efficacy we must use an information parameter from the WF, an important parameter of a probability distribution: the Shannon-entropy (S_{Sh}) [54], as it is discussed in the first part of this series [27]. The SE parameter measures the diversity of probability density function (pdf), which is in the case of Weibull distribution:

$$S_{Sh}\left(n,t_0\right)=\gamma\left(1-\frac{1}{n}\right)+\ln\left(\frac{t_0}{n}\right)+1=S_{Sh1}\left(n\right)+S_{Sh2}\left(t_0\right) \tag{17}$$

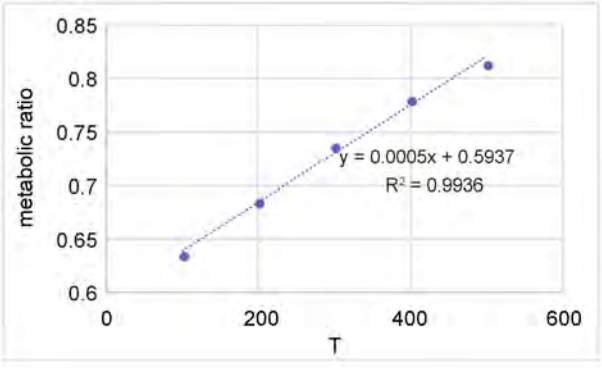


Figure 12. The metabolic ratio (approximate *SUV* ratio) vs. *T* is late start time.

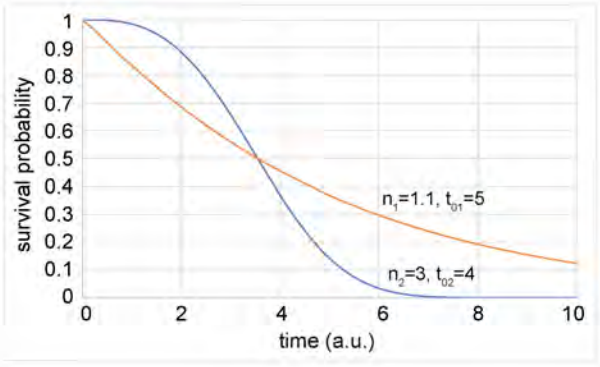


Figure 13. The two-survival function has the same median (=3.54). However, the survival curves are very different ($n_1 = 1.1$, $t_{01} = 5$; $n_2 = 3$, $t_{02} = 4$), which treatment is more effective? Shannon entropy decides.

where γ is the Euler-Mascheroni constant: $\gamma \cong 0.5772$, and

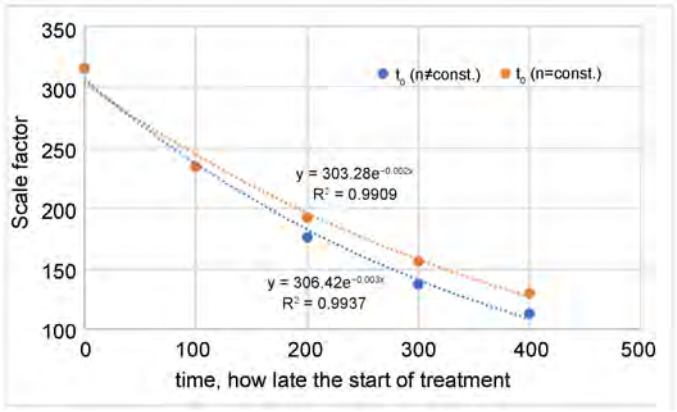
$$S_{Sh1}(n) = \gamma\left(1 - \frac{1}{n}\right) - \ln(n) + 1; \; S_{Sh2}(t_0) = \ln(t_0) \tag{18}$$

The information source of S_{Sh} is produced by a stochastic data-source, like the probability distribution of the survival time. In the simple formulation, it refers to the amount of uncertainty about an event associated with a given probability distribution. At the probability of the survival, this directly means, that the decreasing entropy shows the increasing probability of death. The easiest way to decide the advantage of a treatment which changes the parameters of the WF, is with this parameter, because the survival is better when S_{Sh} is higher. It is due to the meaning of the entropy: a larger entropy means less information and a higher uncertainty of death. Visualizing it on the image of the pdf, it has more located peak when n grows, and its width is shrinking by t_0 , therefore both make death more definite. The growing n and decreasing t_0 both decrease the entropy, making the certainty of death higher. In the case of **Figure 13**, the entropies are $S_{Sh1} = 1.67$ and $S_{Sh2} = 2.58$, consequently the survival with $n_2 = 3, t_{02} = 4$ parameters is worse than the survival characterized by $n_1 = 1.1, t_{01} = 5$.

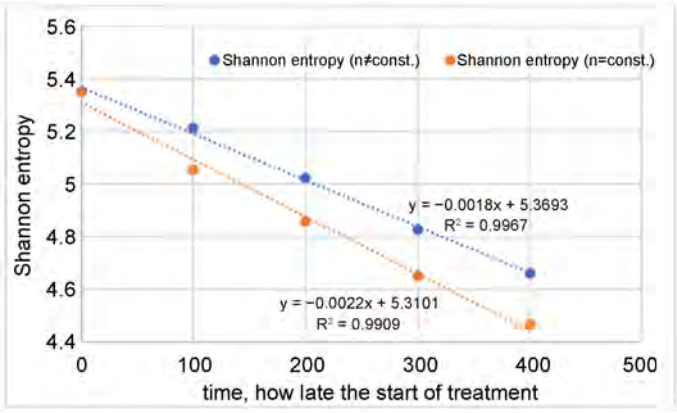
The entropy evaluation in the case shown in **Figure 7** is presented in **Figure 14**. The lower chance of survival is shown well by the decrease of the entropy with the late start times (T_i). This is complete correspondence with the expectations: the later cancer diagnosis decreases the prognosed survival.

Interestingly, despite the more moderate decrease of the scale factor when the shape factor decreases in optimal fit, the Shannon entropy shows an advantage for these optimal WF sets, compared to the constantly fixed shape. The reason is that the patients with longer survival time are fit for the later start of the treatment and were selected by their other, less hazardous conditions than the others.

The Shannon entropy can be evaluated for late-start treatments (treatments in various stages of the tumor) like that it is shown in **Figure 9**. The Shannon entropy



(a)



(b)

Figure 14. The scale factor and the Shannon entropy in the stages of late treatment time shown in **Figure 7**. (a) The scale factors, (b) Shannon entropy values.

for non-responding patients (group A), and for responding ones (group B) is shown in **Figure 15**. The decrease of the entropy well shows the increasing certainty for events.

The Shannon-entropy decreases the number of patients at risk linearly, due to the increasing certainty of death (**Figure 16**).

We assume, that no extra comorbidity developed (or at least it is controlled) over the elapsed time, consequently, we kept the original two parameters (shape and scale) unchanged, regarding the same cohort of patients participated; only their study started in different F_i times. When we calculate with the developing comorbidities, then both parameters of WF will be changed in a direction that S_{Sh} decreases, indicating a higher certainty of the event.

5. Conclusion

We discussed a method of data mining from the single-arm clinical study without a reference group. We studied the possibility to open the hidden information in the measured Kaplan-Meier non-parametric estimate by the composition of proper parametrization of cumulative Weibull functions. We had shown the

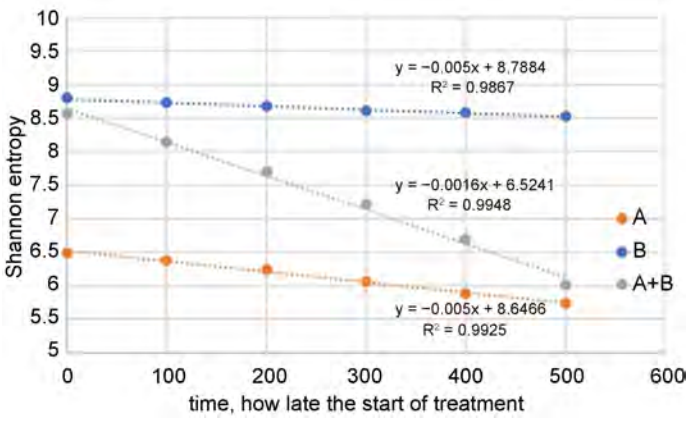


Figure 15. Shannon-entropy decreases in both the non-responding (A) and responding (B) groups of the patients. The change is 10 times more rapid for non-responding group. The composite of the real overall survival (measured *KM*) from these components shows the entropy-change more characteristically (The evaluation is made for the *KM* curves in **Figure 9**).

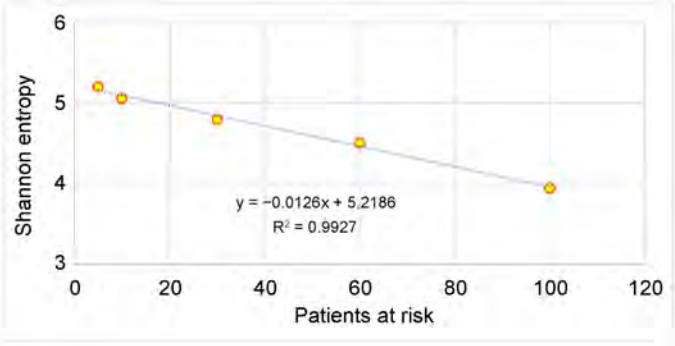


Figure 16. Shannon-entropy decreases by the number of patients at risk (Original WF: $n=2$; $t_0=100$, $N=100$).

changes of the two independent parameters of the Weibull cumulative distribution by the study design, namely their dependence on the inclusion criteria (staging) and the intended end-point (finishing). We had shown that the various studies with different inclusion and exclusion criteria and different endpoints could be well described by the decomposition method. The fit of these results to real studies in clinical applications will be shown in the next part of this series of articles.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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