

A potential new way of defining the risk of malnutrition and its correlation with biological age

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Summary

A potential new way of defining the risk of malnutrition and its correlation with biological age

Malnutrition is a common underdiagnosed condition worldwide both in hospital and outpatient settings. It is associated with higher levels of complications and mortality, poorer outcomes and higher hospital costs. Thanks to various initiatives in the field of nutrition, new screening and assessment methods for the detection of malnutrition are constantly being developed, which are intended to be time-saving and convenient.

However, there is evidence of them being not sufficiently implemented in medical practice. With our pilot study, we hope to develop a new idea to address this problem. The idea of automating nutritional screening seems attractive and could help healthcare facilities around the world not to underestimate the importance of diagnosing malnutrition.

By analysing routinely done blood tests on patients, the results of their nutritional screening and relying on literature data, we have developed a pilot formula that allows us to estimate the risk of malnutrition in a patient. While our results do not yet report the formula results being as sensitive as existing screening or assessment methods, however, it is a promising and prospective direction for the effective diagnosis of malnutrition.

The correlation of malnutrition not only with blood parameters, but also with patient age, was an area of interest to me. Existing studies indicate a more relevant biological age for predicting morbidity and mortality, which differs from the chronological age due to changes at the cellular and molecular level. Assuming that this indicator correlates better with the presence of malnutrition, in the second part of the thesis an analysis of this relationship was performed. The results obtained give the impression of a correlation between biological age and malnutrition, which also opens up new horizons for the research in this field.

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Introduction

Malnutrition in hospitalised patients remains a significant problem leading to more frequent complications, poorer outcomes and increased hospital costs.¹ According to various data, it affects 19-60% of hospitalised patients.² Unfortunately, malnutrition and its associated risk remain a very challenging problem in many health institutions around the world, mainly because of being underdiagnosed and unrecognised.³ The main consequences of malnutrition on physiological functions are the decline in muscle function and muscle mass, reduction in cardiac muscle mass, changes in pancreatic exocrine function, intestinal blood flow, psychosocial effects, etc.⁴ All of these have a significant impact on clinical outcome. Malnutrition in healthcare facilities can stem from a variety of factors, some of the main reasons are disease-related problems (decreased appetite, difficulty eating, or increased nutritional requirements), limited choice of hospital food, malabsorption/maldigestion problems, inadequate training or awareness of the importance of nutrition among healthcare staff, etc.⁵

Many screening and assessment tools to detect malnutrition have been created, each with its own advantages and disadvantages. However, in practice, the importance of their performance is either underestimated or possibly there is a lack of knowledge about malnutrition in a healthcare facility in general, as it was mentioned above.⁶

The structure of screening and assessment tools (MUST, NRS-2002, GLIM, SGA) was designed in such a way that their use was the least time-consuming for medical personnel, although human factors plays a

role, and it is still no tool of choice or gold standard that is recognised worldwide as the most effective. However, there are numerous studies comparing different screening and assessment methods and many of them agree that SGA is the most sensitive method.^{7,8}

However, SGA performance requires effort and time, perhaps not much for an individual patient, but in the case of a mass assessment of the entire ward, department or hospital, it can be very time consuming.

How else can malnutrition be detected, without taking up medical staff time and still being strong enough to detect malnourished patients? Due to the above, the idea of automating the detection of malnutrition could be very helpful and perspective. Automated screening/assessment tools could work quickly and efficiently with a large number of patients, helping healthcare staff to identify individuals at risk of malnutrition in a timely manner. This would lead to earlier interventions and improved outcomes. However, the implementation of malnutrition screening scales usually requires an interview with each patient so their performance is unlikely to be automated and performed by, for instance, a computer or AI.

In contrast to malnutrition screening tools, blood tests are routinely performed on almost all inpatients and we know that some blood parameters can be correlated with malnutrition. Therefore, the system of detecting malnutrition using some blood parameters could be a time-saving and effective way to solve the problem. However, the existence of a single parameter that reflects the risk of malnutrition is still questionable. Therefore, we came up with the idea of considering the combination of blood parameters that could reflect the risk or presence of existing malnutrition.

This thesis additionally analyses an idea of malnutrition correlates not only with blood parameters, but also with the patients' age. It is also worth noting that there is currently a perspective of defining not only a chronological age but also a biological one, and that this theory is now widely studied. The following investigation of a possible correlation between biological age and malnutrition is presented in Part 2 of this thesis.

Background

Talking about biochemical markers of malnutrition, albumin is probably the most common and well-studied. Some screening tools for malnutrition such as the Prognostic Nutrition Index (PNI) include albumin in the list of parameters to be determined.⁹ However, it is now widely reported that albumin has a rather low specificity in detecting malnutrition and can reflect a general inflammatory process and the severity of the disease.¹⁰ It has been reported that not only albumin, but also other blood parameters are correlated with the patient's nutritional status.¹¹ However, since a review of the literature revealed that not only albumin is correlated with nutritional status the idea of considering several blood test parameters simultaneously to detect malnutrition seems reasonable.

Though, the similar idea has been discussed a very long time ago, even in 1979 Seltzer et al had proposed using a combination of 2 common blood tests to «form the basis of instant nutritional assessment»¹² paralleled by a

later similar proposal from a Glasgow oncology group.¹³

The key question to be discussed was the selection of valid blood parameters that could possibly be which are potentially associated with the presence of malnutrition. Probably, the most commonly described blood test in this context is albumin: hypoalbuminemia is known as a possible marker of malnutrition.

Some studies have shown a correlation between serum albumin levels and anthropometric parameters; in geriatric patients, albumin levels have been found to correlate with MNA risk score.¹⁴ However, as mentioned above, abnormal levels are not necessarily indicative of malnutrition: a meta-analysis concluded that albumin levels must be interpreted cautiously in the presence of an inflammatory process is in presence,¹⁵ as they may be more indicative of recent acute stress.¹⁶ Nevertheless, the presence of inflammation and disease carries the risk of malnutrition. Again, serum albumin levels could be useful in an automated multiparameter malnutrition screening tool.

Talking about inflammatory markers, not only albumin is an available parameter to reflect the inflammatory process. If malnutrition correlates with the presence of inflammation, it is logical to assume that other inflammatory markers could also be helpful. In addition, disease-related inflammation with its physiological effects such as insulin resistance and decreased appetite, is a common underlying driver of malnutrition.¹⁷

C-reactive protein as a positive acute-phase reactant, whose levels are elevated in both acute and chronic inflammation, is used as an objective measure to define inflammation due to its high sensitivity and

availability¹⁸ in serum. However, the meta-analysis did not provide a basis for the claim that CRP correlates with screening tools (MNA, SGA, NRS-2002, GNRI), but only showed that acute disease stress affects serum levels of some biomarkers (especially CRP).¹⁵ But there is evidence of an association between lower dietary intake and inflammatory status as measured by C-reactive protein levels, suggesting that the risk of malnutrition may also be observed with elevated CRP levels.¹⁹

Talking about other signs of markers of inflammation, some correlation of leucocyte activity and malnutrition has been described, with decreases in the phagocytic and chemotactic activity of neutrophil, but this information is not accessible from the routine full blood count/picture.²⁰

There is reason to believe that red blood parameters such as haemoglobin, hematocrit and red blood cell count are also related to nutritional status. In addition, anaemia is a fairly common condition in hospitalised patients and is associated with many chronic diseases. Also, red blood cell parameters are usually included in any routine blood test, so they are interesting as an element of an automated scale.

Haemoglobin is one of the most commonly studied blood biomarkers, but studies disagree on whether it can accurately predict nutritional risk. Some studies show no significant correlation between dietary risk and lower haemoglobin levels. However, a meta-analysis concluded that haemoglobin levels were significantly lower in individuals at high risk for malnutrition than in those not at risk.¹⁵

Moreover, there is evidence of an association between sarcopenia and anaemia²¹, and sarcopenia is a common condition in malnutrition. Also, according to some studies,²² lower levels of red blood cells (RBC) among

elderly were more prevalent in malnourished subjects compared to well-nourished subjects.

Another group of parameters that are routinely checked in blood tests are the blood electrolytes. Their relationship with nutritional status is not well known in the literature. However, correlations have been demonstrated between a simplified creatinine index and nutritional risk in older people.²³ Associations have also occasionally been shown between abnormal serum electrolytes and nutritional status, such as from the malnutrition inflammation score in chronic kidney disease²⁴, or in outcomes from severe acute malnutrition in children.²⁵

Thus, many of the blood parameters that are normally determined during routine tests can serve as a good basis for the creation of a new automated score that can be used to quickly and effectively determine the risk of malnutrition in patients. However, as shown in the literature, no blood markers can reflect the presence of malnutrition specifically enough, but they mostly reflect general morbidity. Given that malnutrition is often underdiagnosed, the primary goal of screening methods is sensitivity rather than specificity: this applies both to existing screening scales (e.g. MUST) and to our idea of developing an automatic scale.

It is possible that the idea of screening based only on blood parameters is not specific for malnutrition, but it is worth considering that at least the risk of malnutrition may be associated with certain changes in blood counts. Therefore, screening for malnutrition risk may be an even more important goal of our pilot project than screening for malnutrition itself.

Methodology



This study and data analysis were carried out using information on 300 patients of the Internal Medicine Clinic of Tartu University Hospital, collected over a 7 month period from September 2021 to March 2022. Patients of an emergency admission and planned admission were both included.

The study was approved by the Research Ethics Committee of the University of Tartu and the Tartu University Hospital Reference 340/T-15. It is registered with <http://clinicaltrials.gov/> as Protocol PKL-127 reference NCT 0554 6034. All participants received an information sheet together with the consent form about the study and contact details of the research team. Data was gathered with full regard to confidentiality of the individual patient.

Patients were enrolled from the Gastroenterology, Rheumatology, Endocrinology and Nephrology departments, the information about their admission and presence was known from the electronic hospital database. Also their diagnosis was taken from the database using the ICD-11 code. The first plan was to collect approximately the same number of patients from each department, but in Rheumatology and Endocrinology, the total number of patients was recruited as in Neurology and Gastroenterology separately, so 3 batches with relatively the same number of patients were formed.

Surely, during 7 months the number of admitted patients in these wards was much bigger than 300, so the exclusion criteria were also followed.

Exclusion criteria were:

- 1) 6 days passed from hospitalisation
- 2) Pregnant
- 3) Lactating patients
- 4) Younger than 18 years
- 5) Surgery, dialysis or other procedures that would change the blood tests before 48 hours of the current hospitalizations first blood tests
- 6) Isolation patients put into contact isolation (COVID, ESBL, VRE, MRSA) were removed from potential interview list, but were entered into it again if their isolation ended and they still had less than 6 days from hospitalisation

After defining which patients meet the criteria to be included, the interviewing process was initiated. Patients were asked if they consent to the usage of their personal data for the study and signed a consent form with the option of having its copy, also their questions were answered. Refusal to participate was an acceptable answer and the total number of refusals was written down in the notes. Patients were asked about having any of the aforementioned exclusion criterias.

Language barriers were not encountered.

During the interview patients were asked open-ended questions to gather data, which included their overall state of health, age, gender, weight, and height, severeness of illness and their own understanding of their diagnosis, dietary changes before admission, and weight loss. During the interview there were determined NRS-2002, Malnutrition Universal Screening Tool (MUST) and Subjective Global assessment (SGA) scores. These three provided the screening data necessary for Global Leadership

Initiative on Malnutrition (GLIM) score. Questionnaire components were pre-set before enrolment into the study was started.

If the patient had no recollection of current weight or the weight given was too old to be of any use in calculating weight loss, then the weight was measured using the scales used in the respective wards. No calibration of the ward scales was done as this was assumed to be done by the wards.

Each patient was recorded to the dataset using a study identification number that was used to make the dataset anonymous but still trackable for the researchers. The consent forms were also marked with the same study identification number in a removable fashion, so that after the removal of them no way remained of identifying the patients in the dataset and anonymity could be guaranteed, all the patients were also informed about that.

No additional interventions were performed.

No revisits were performed after initial interviews.

Statistical analysis

We recruited 300 patients, this set was then divided using Excel Microsoft office into two groups: a training set of 200 patients and a final set of 100 patients. Blood tests had been performed in all 300 patients, mostly simple haematological and biochemical parameters, which are common to be done as a standard blood tests among all inpatients. In total 88 different blood test parameters were recorded.

Choice of exact blood tests for the following work and study was one of

the key questions. Since one of our main ideas was to use those tests that are routinely performed on most patients, we first excluded all parameters from the training set that were performed infrequently, choosing only those which were performed in at least 60% of the patients, thus leaving us with 37 of tests. The remaining variables were then subjected to factor analyses to examine whether these could be reduced to less dimensions.

For this the missing data was imputed with the median of the variables, which is considered a robust imputation method and in order to allow for the production of a factor score that could be tested in a repeat clinical practice, the variables were normally standardised.

Statistical characteristics of these blood tests for the training and validation sets can be found in the Appendix.

The next step was to develop a suitable multivariate logistic regression (MLS) model that would be able to predict each nutrition screening tool. We decided that the best method was to run stepwise logistic regression with backward elimination based on the likelihood ratio. A backward elimination (based on p-value of the predictor removed) was chosen over forward selection, based on our preference to be able to include as many variables as possible in any possible model developed, of course also satisfying the principle of parsimony. The variables chosen for MLS were based on the variables that had more than 60% of values available and were significant in the univariate logistic regression analyses, to remain consistent with the approach in the factor analysis.

For the secondary outcomes we made the simple comparison of SGA being the dependent variable and the binary variables of NRS-2002 and MUST being the predictors.

Results

From 337 patients approached, 300 were eligible as a final sample with 52% female. Average age was 60 ± 17.7 SD y.o., average BMI was 27.8 ± 6.32 SD. Average weight was 80.1 ± 20.1 kg. Their diagnoses were categorised by ICD-11 with following codes (groups of diseases): K (29%), M (20%), E (13%), D (10%), N (9%).

By using univariate and then, multivariate analysis, as it was said above, 37 of the most common blood tests were analysed, compared to SGA results as the “reference” method for assessing malnutrition. Stepwise logistic regression method was used, as a result, it turned out that In patients with every increase in 1 unit of monocytes and neutrophils (%), patients were 12.36 and 1.09 more likely to have moderate or severe malnutrition. Next, in patients with every increase in 1 unit of neutrophils, potassium and albumin, patients are 0.77, 0.33, and 0.87 less likely respectively to have moderate or severe malnutrition. The predictive equation of the model and the formula to calculate the new score was

$$p(Y = 1 | X) = \frac{1}{1 + e^{-\left(4.266 + 2.514 \times (\text{monocytes}) - 0.259 \times (\text{neutrophils}) + 0.085 \times (\text{neutrophils } (\%)) - 1.112 \times (\text{potassium}) - 0.138 \times (\text{albumin})\right)}}$$

Hereby is a ROC plot for the model trained on training set vs SGA:

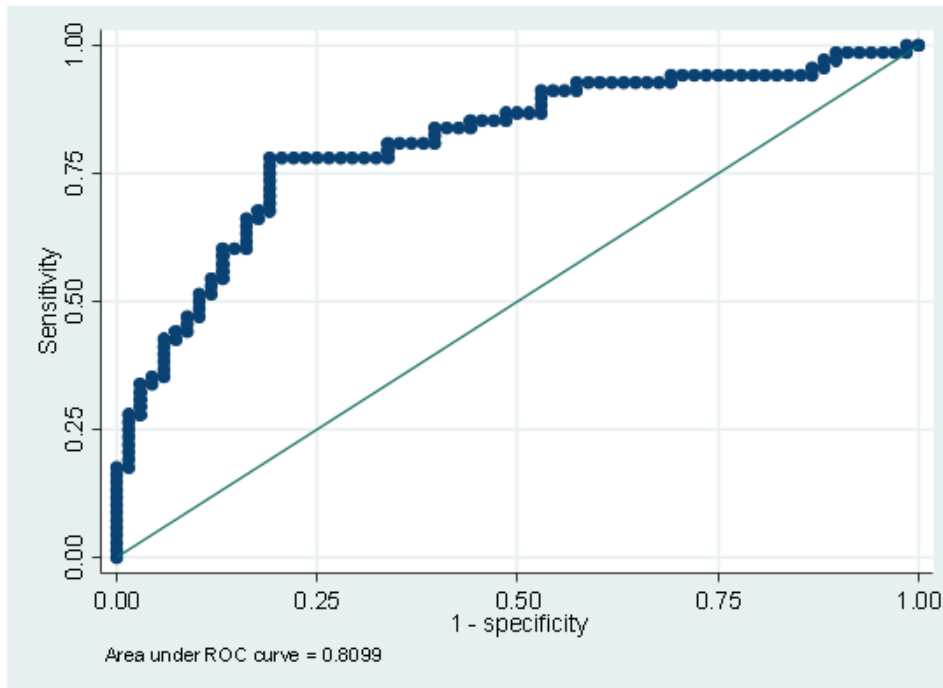


Figure 1. ROC plot for the model trained on training set vs SGA

The model as a whole explained between 16% (McFadden's Adjusted R²) 27.4% (Cox and Snell R square) and 36.5% (Nagelkerke R squared) of the variance in nutritional risk, and correctly classified 78.4% of cases having an area under the ROC of 0.810 .

The model has largely a satisfactory fit. The Hosmer–Lemeshow or Pearson χ^2 goodness-of-fit tests were non-significant and AIC 1.165 and BIC -492.263 indicating satisfactory fit.

Using the above calculation formula, our new score value was obtained, in most cases, from 0 to 1. Next, the question arose of what value in this range would be the threshold for determining the risk of malnutrition. For this, the analysis of different sensitivity and specificity indexes was used. The process of it is shown in Table 1.

	true positive	false positive	sensitivity	true negative	false negative	specificity	acc
0.53	10	17	37%	39	11	78%	0.49
0.45	26	26	50%	31	17	65%	0.57
0.5	24	24	50%	33	19	63%	0.57
0.55	23	16	59%	41	20	67%	0.64
0.6	21	14	60%	43	22	66%	0.64
0.65	17	13	57%	44	26	63%	0.61
0.7	17	12	59%	45	26	63%	0.62

Table1. Cut off values of validation set 

However, when this model was tested on the training and validation set, ROC curves showed less sensitivity and specificity (Figures 1 and 2).

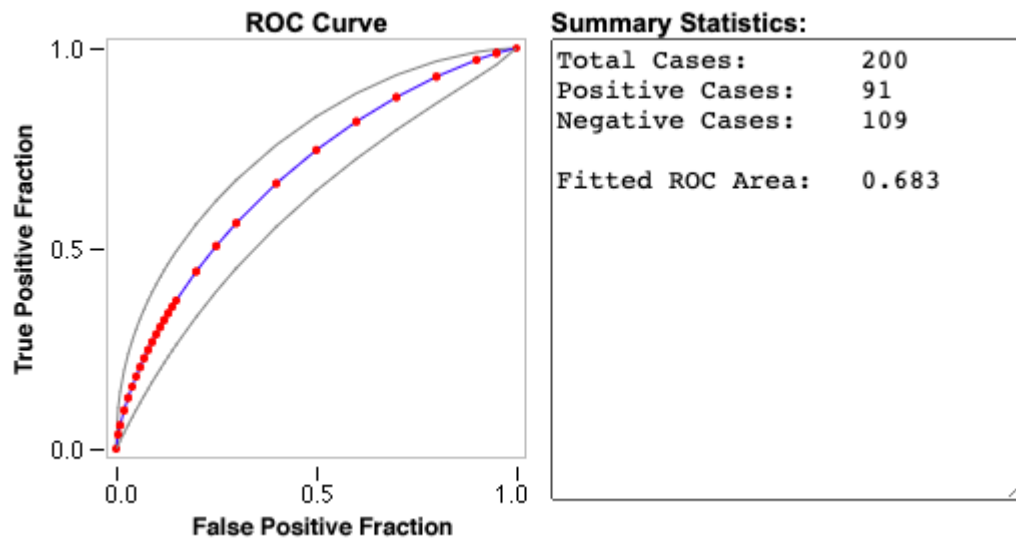


Figure 2. ROC curve for SGA and the new score

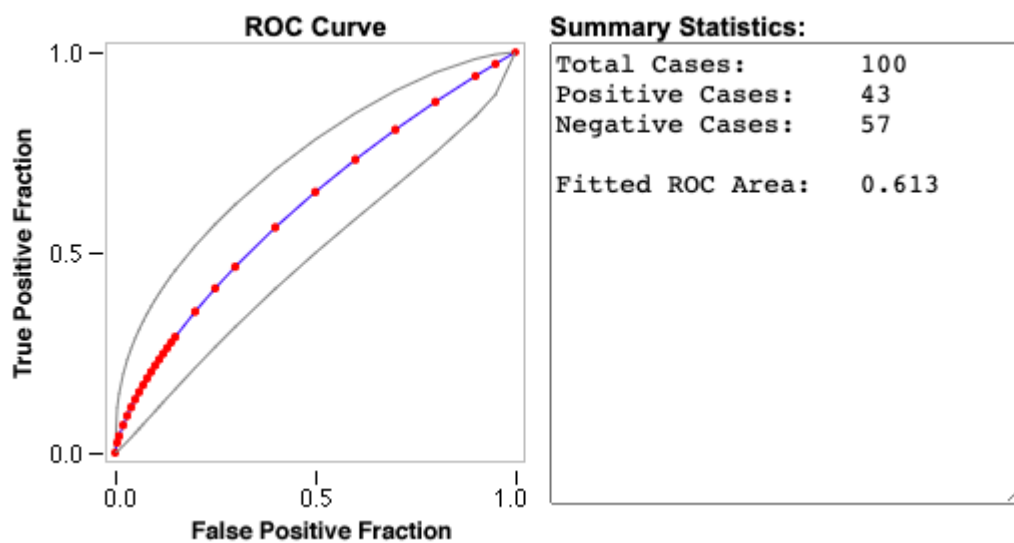


Figure 3. ROC curve for SGA and the new score (100 validation set)

Also the performance of MUST and NRS-2002 to predict SGA was studied:

		SGA	
		yes	no
NRS cut off values 3	yes	40	5
	no	3	52
	sensitivity	93%	
	specificity	91%	
	acc	92%	
		SGA	
		yes	no
MUST cut off value 2	yes	27	5
	no	16	52
	sensitivity	63%	
	specificity	91%	
	acc	79%	
		SGA	
		yes	no
MUST cut off value 1	yes	35	7
	no	8	50
	sensitivity	81%	
	specificity	88%	
	acc	85%	

Table 2. Performance of MUST and NRS-2002 to predict SGA in the validation set

Discussion

In general, it can be said that the following work and investigation of our model is needed. However, our results are promising  and there is a perspective of the future using of it and integrating into a global clinical practice.

Based on our results, the model's performance in predicting SGA is worse overall when analysing the ROC curves.

The sensitivity compared to the MUST cut-off value 2 is 16% better in the training set but 3% worse in the validation set. For the MUST cut-off value 1, sensitivity is 9% better in the training set and 21% better in the validation set. 

In comparison, the sensitivity for NRS-2002 is 10% worse in the training set and 33% worse in the validation set. Despite this, the model's specificity for SGA results in lower overall accuracy compared to MUST and NRS-2002, as SGA remains a crucial indicator for identifying malnutrition.

There may be several reasons for this result: first, our sample size is relatively small: most nutritional screening studies have a much larger number of patients recruited. Also, even the most frequently performed blood tests (37 parameters) were not completely collected from all patients in this sample. In this case, the missing data was replaced by the mean value. This can potentially severely affect the final result; for the best possible result of the experiment, it is necessary to use only “real” parameter values.

Nevertheless, the potential of a new automatic screening tool is promising. Even with our current results, if this score were a universal method for detecting malnutrition or its risk, it would save a lot of time: If it takes at

least 4 minutes (often more) to perform the SGA, it would take 1200 minutes to screen all patients, totaling 20 hours. Our new score can potentially be calculated automatically in a few seconds using a computer. If this function is performed by default, e.g. after receiving the test results into the hospital's internal electronic system, such screening will be performed for all patients without exception, whereas currently nutritional screening can easily be overlooked in some patients for various reasons. The strengths of this study are: a completely new approach to screening for malnutrition (combining blood tests to produce an automatic calculation is a new way to define malnutrition), the use of several established screening methods to obtain and validate the data, and the comparison of the test set with the validation set.

However, there are some limitations: The method should be used with caution in certain categories of patients in whom one or another blood test indicator used in the calculation formula deviates unusually from the norm: For example, in a patient diagnosed with acute leukaemia, the new score value was over 100, whereas in most patients this value is between 0 and 1. Certainly, even this approach is not yet applicable in paediatric practice. Future studies could help to solve these problems.

Conclusions:

1)The use of a new score is a very promising approach in determining the nutritional status of hospitalised patients. Even with the current statistical analysis, sensitivity and specificity of it are comparable to existing malnutrition screening scales and can be used in the future as a new approach to screening malnutrition.

2)This study is a pilot one, though, future studies with bigger patient samples and more centres involved are required to develop and improve our new approach. This could potentially help with the recognition of malnutrition globally and make screening time-saving and simple.

Part 2

Biological versus chronological age and the presence of malnutrition

Introduction

During this academic year, I have not only been involved with the new automatic screening tool, but also with research into another parameter that is also important for malnutrition. During my internship in Rome under the supervision of Prof. Alessandro Laviano, Associate Professor of Internal Medicine at the Department of Clinical Medicine of the Sapienza University of Rome, Italy, I conducted a side project looking at the correlation of malnutrition and biological age of patients. It is not yet ready to be formalised as an independent work or project, but nevertheless these thoughts deserve to be shared in this paper, as they can serve as a promising vector for the development of this topic in the future.

It is well established that malnutrition is more common in older patients due to various physiological and social aspects as well as the high prevalence of comorbidity and disease-related malnutrition.²⁶ Not only nutritional status but also morbidity in general is mostly age-related and can be explained at the cellular level: altered gene expression, mitochondrial dysfunction and metabolic variations. However, chronological age itself may not be the only and not the best reason for this: Kwok M. et al. (2023) reported that biological age is superior to chronological age in predicting mortality and morbidity.²⁷ Biological age can influence how individuals respond to medical treatments and

interventions. People with an older biological age may have a lower capacity for repair and recovery, which affects the effectiveness of therapies and interventions to improve health. DNA methylation-based estimators correlate well with chronological age and are also a potential predictor of accelerating biological age.²⁸ However, analysis of differently methylated DNA regions is not a common test to be performed globally and routinely. Morgan E. Levine et al. (2018) suggested an estimation of biological age based on selected clinical blood test parameters and chronological age.²⁹

We hypothesise that the presence of malnutrition might correlate better with biological rather than chronological age. One of our aims was to investigate the correlation between the presence of malnutrition and biological age acceleration. To assess the risk of malnutrition, we used 4 well-known screening and rating scales: MUST, NRS-2002, GLIM and SGA in 300 patients. For the estimation of biological age, we used a calculation based on clinical blood test parameters and chronological age from Levine et al. In general, the correlation between biological age and the presence of malnutrition was either significantly mildly positive or significantly moderately positive. The choice of a screening scale for malnutrition is still a question that requires further research and discussion. However, the risk of malnutrition defined by using the NRS-2002 scale shows a slightly higher correlation with the presence of biological age acceleration.

Scientific background

Older human age is widely associated with higher morbidity. Age is a common risk factor for many diseases:³⁰ chronological age is included in many medical scores and calculations which predict risk of various events, conditions and states (SCORE, HEART, CHA2DS2-VASc, etc). Ageing is also correlated with malignant processes in human organisms: most cancers arise in individuals over the age of 50-60.³¹ Declining cognitive function, changes in hormonal status, increased inflammation — all these factors increase the risk of chronic and acute diseases.

The correlation of nutritional status with age is also one of the key issues in clinical nutrition practice: malnutrition is much more common in older patients, both due to the physiological characteristics mentioned above and to psychosocial factors.²⁶ Age also is a part of some existing malnutrition screening tools, e.g., NRS-2002.

The reason underlying the physiological decline and higher morbidity lies in changes in tissue, cellular and molecular processes in the body. A number of them have been discovered in the last 100 years: telomere and mitochondrial dysfunction, loss of proteostasis, stem cell exhaustion, and epigenetic alterations like spontaneous mutations.³² Many pathways of ageing are also associated with chronic oxidative stress.

Recently, however, the terms «chronological» age and «biological» or «phenotypic» age have often been used. Chronological age is only a simple calendar parameter, whereas biological age is determined at the molecular level (based on the above-mentioned signs of tissue and cellular ageing) and may differ upwards or downwards from chronological age.

The concept of «epigenetic clocks», which are supposed to reflect the «true» biological age of humans, has become widespread among researchers. DNA methylation is a key factor that reflects the functioning of the aforementioned biological epigenetic clock. Therefore, many models for determining biological age have been created based on this indicator: the model by Bocklandt et al. in 2011, by Hannum et al. and Horvath et al. in 2013, etc.^{33,34} However, these «clocks were relatively weak in mortality prediction, which should have been a reasonable reflector of «biological» age. Thus, more functionally relevant molecular changes have been explored. Zhang et al. used LASSO Cox regression to identify 10 DNA sites that were highly correlated with mortality risk,³⁵ and, based on this study, Levine et al. created the DNAm PhenoAge clock, which uses predicts a composite biological age score based on a linear combination of chronological age and nine clinical parameters associated with mortality risk.²⁹ DNAm PhenoAge is a pilot synonym for a «biological» age, as it was reported in the reference. Predicted estimates from the Levine et al. model represent a person's epigenetic age, which they refer to as 'DNAm PhenoAge'.

For this reason, the concept of this clock was interesting for my thesis, as it uses the model of predicting age status based on commonly performed blood tests. This was not the only reason, but also the fact that age and risk of malnutrition correlate with each other, and it is reasonable to assume that not only morbidity and mortality can be better predicted with PhenoAge, but also the risk of malnutrition. Since we have a database of 300 patients and their nutritional screening and assessment results, we can even test different malnutrition scales for their correlation with biological and chronological age.

Thus, writing this part of the thesis, the following aims were set:

- 1) Determine and evaluate the relationship of the presence of malnutrition to the age of patients (both chronological and biological).
- 2) Determine which of the screening/assessment tools used was more strongly associated with patients' chronological and biological age.

Methodology

After analysing the scientific background, it was decided that the primary database of 300 patients (training+validation set) used for the abovementioned «Tartu score» (or new score) study is suitable for primary analysis of the correlation of biological age with the presence of malnutrition. For the detection of PhenoAge of each patient following inputs (described by Levine et al.) are required: chronological age and 10 following blood test parameters: albumin, creatinine, glucose, C-reactive protein, lymphocyte percent, mean cell volume, red cell distribution width, alkaline phosphatase, white blood cell count. These inputs were known for all 300 patients in the database. The missing data was imputed with the median of the variables, which is considered a robust imputation method and in order to allow for the production of a factor score that could be tested in a repeat clinical practice, the variables were normally standardised. If any of the required blood test parameters result was missing, it was replaced by the median of existing results.

All the calculations of the PhenoAge estimation were performed according

to the Levine et al. study, including the main PhenoAge estimation formula:

$$\text{CDF. univariate}(t, \text{age}) = 1 - e^{-\{ -e^{(\text{age} * b_1 + b_0)} \gamma - 1 (e^{\gamma t} - 1) \}}$$

$$\text{PhenotypicAge}_j = 141.50225 + \frac{\ln(-.00553 * \ln(1 - \text{MortalityScore}_j))}{0.090165}$$

This formula was put into Excel dataset Version (Build 16227.20258 Click-to-Run) so the estimated PhenoAge was able to be calculated automatically. After fixing all the PhenoAge results it was decided to compare the correlation of presence of malnutrition (according to the result of each of the scales) with PhenoAge and the correlation of malnutrition with the generally used chronological age.

The total patient dataset was 300, which was also randomly split using randomly generated numbers by Excel Version (Build 16227.20258 Click-to-Run) into 200 for training and 100 for validation of generated algorithms (as in the main part of this thesis). For the correlational analysis the Spearman correlation coefficient was chosen and online software for its calculation was used (<https://www.socscistatistics.com/tests/spearman/default2.aspx>). Two sets of patients (100 and 200) were analysed separately from each other.

No additional interventions or interviews were performed.

Results

By analysing the whole sample of 300 patients, the percentage of female gender was 52%. Mean chronological age was $60 \pm 17,6$ y.o. Their mean PhenoAge was $76 \pm 28,2$ y.o.. Mean difference between two values (PhenoAge and chronological age) was $16,05 \pm 22,3$.

By using Levine et al. PhenoAge calculation, the maximal possible PhenoAge value was 123,83 y.o., as their system produces a technical error when working with larger numbers.

214 of 300 patients (71%) had the value of PhenoAge bigger (older) than their chronological age.

Then, starting with the first sample (200 patients), Spearman's correlation analysis gave the following results:

	Chronologic age		PhenoAge	
	R	p value	R	p value
MUST	0.0523	0.471	0.155	0.032
NRS-2002	0.3	<0.001	0.298	<0.001
GLIM	0.0612	0.399	0.164	0.023
SGA	0.16	0.027	0.203	0.005

Tab.3 Correlation between the presence of malnutrition according to different screening/assessment tools and chronological with biological age (200 test set).

The next test sample of 100 patients was also analysed by using Spearman's correlation with the following results:

	Chronologic age		PhenoAge	
	R	p value	R	p value
MUST	0.053	0.601	0.215	0.032
NRS-2002	0.231	0.021	0.317	0.001
GLIM	0.0608	0.548	0.169	0.092
SGA	0.016	0.874	0.21	0.036

Tab.4 Correlation between the presence of malnutrition according to different screening/assessment tools and chronological with biological age (100 test set).

It has been suggested that it is not the value of chronological or biological age itself that may be associated with the presence of malnutrition or its risk, but rather the difference between these two values in a particular patient. This parameter was also calculated and its relationship with the presence or absence of malnutrition was analysed according to all tested tools.

	PhenoAge value - Chronologic age value	
	R	p value
MUST	0.256	<0.001
NRS-2002	0.263	<0.001
GLIM	0.219	0.002
SGA	0.223	0.002

Tab.5 Correlation between the presence of malnutrition according to different screening/assessment tools and two types of age numerical difference (200 test set)

	PhenoAge value - Chronologic age value	
	R	p value
MUST	0.265	0.008
NRS-2002	0.275	0.006
GLIM	0.144	0.153
SGA	0.272	0.006

Tab.6 Correlation between the presence of malnutrition according to different screening/assessment tools and two types of age numerical difference (100 test set)

Discussion

When analysing the mean chronological and biological age, the percentage of patients with a PhenoAge greater than the actual age was quite high (71%). This can be explained after a careful evaluation of the parameters used in the PhenoAge calculation formula. In general, it can be said that the PhenoAge calculation can be considered similar to the calculation of the severity of the patient's condition. Higher values for creatinine, glucose, C-reactive protein, mean cell volume, red blood cell distribution width, alkaline phosphatase, white blood cell count, and lower values for percentage of lymphocytes and albumin are associated with a higher PhenoAge score. It should also be remembered that chronological age is factored into the formula, i.e. two patients with the same blood values but different ages will have different PhenoAge values. The fact that biological age was widely used in our sample is explained by the fact that the calculation of PhenoAge is based on biochemical parameters that are often due to disease-related changes in hospitalised patients, and is absolutely consistent with the idea that PhenoAge can serve as a good predictor of worse outcomes and higher mortality. However, it is still unclear how large the increase in biological age should be to predict possible worse outcomes.

It is worth noting that the results of the statistical analysis of the two samples (200 and 100) are statistically relatively similar. In the correlation analysis, both age groups (chronological and biological) mostly have a weak positive relationship with the results of the screening tools for malnutrition. However, most of the relationships with biological age

(MUST, NRS-2002, SGA) were significant (which is usually not the case for chronological age).

The relationship between screening results using the NRS-2002 deserves an additional discussion. The correlation coefficient and p-value when comparing the results of this scale with both types of ages turn out to be statistically more strong and significant than similar indicators obtained when comparing with the results of other tools. Perhaps it can be explained with the age parameter included into the NRS-2002 criteria.

The correlation of the presence of malnutrition or its risk with the presence of an increase in biological age is also positive and statistically significant (except for the correlation with the GLIM scale).

Conclusions

In conclusion, while the use of biological age as a complementary measure to chronological age can provide valuable insights into general health and ageing processes, it is important to consider the complexities, limitations, and potential psychological implications of relying solely on biological age assessments. Considering both chronological and biological age can lead to a more holistic understanding of a person's health and current condition.

Literature references

- 1) Marinos Elia, Nutrition and health economics, Nutrition, Volume 22, Issue 5, 2006, Pages 576-578, ISSN 0899-9007
- 2) Stratton RJ, Hackston A, Longmore D, Dixon R, Price S, Stroud M, et al. Malnutrition in hospital outpatients and inpatients: prevalence, concurrent validity and ease of use of the 'malnutrition universal screening tool' ('MUST') for adults. Br J Nutr. 2004 Nov;92(5):799–808
- 3) Bellanti F, Lo Buglio A, Quiete S, Vendemiale G. Malnutrition in Hospitalized Old Patients: Screening and Diagnosis, Clinical Outcomes, and Management. Nutrients. 2022 Feb 21;14(4):910. doi: 10.3390/nu14040910. PMID: 35215559; PMCID: PMC8880030
- 4) Saunders J, Smith T. Malnutrition: causes and consequences. Clin Med (Lond). 2010 Dec;10(6):624-7. doi: 10.7861/clinmedicine.10-6-624. PMID: 21413492; PMCID: PMC4951875
- 5) Brglez M, Plazar N, Poklar Vatovec T, Meulenberg CJW. Health concerns regarding malnutrition among the older populations: considerations from a Slovenian perspective. Health Promot Int. 2022 Feb 17;37(1):daab097. doi: 10.1093/heapro/daab097. PMID: 34179974
- 6) Traub J, Reiss L, Aliwa B, Stadlbauer V. Malnutrition in Patients with Liver Cirrhosis. Nutrients. 2021 Feb 7;13(2):540. doi: 10.3390/nu13020540. PMID: 33562292; PMCID: PMC7915767
- 7) Sohrabi Z, Kohansal A, Mirzahosseini H, Naghibi M, Zare M,

- Haghighat N, Akbarzadeh M. Comparison of the Nutritional Status Assessment Methods for Hemodialysis Patients. *Clin Nutr Res*. 2021 Jul 20;10(3):219-229. doi: 10.7762/cnr.2021.10.3.219. PMID: 34386441; PMCID: PMC8331289
- 8) Du H, Liu B, Xie Y, Liu J, Wei Y, Hu H, Luo B, Li Z. Comparison of different methods for nutrition assessment in patients with tumors. *Oncol Lett*. 2017 Jul;14(1):165-170. doi: 10.3892/ol.2017.6154. Epub 2017 May 11. PMID: 28693149; PMCID: PMC5494893
 - 9) Buzby GP, Mullen JL, Matthews DC, Hobbs CL, Rosato EF. Prognostic nutritional index in gastrointestinal surgery. *Am J Surg*. 1980 Jan;139(1):160-7. doi: 10.1016/0002-9610(80)90246-9. PMID: 7350839
 - 10) Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *Int J Gen Med*. 2016 Jul 15;9:229-55. doi: 10.2147/IJGM.S102819. PMID: 27486341; PMCID: PMC4956071.
 - 11) Keller U. Nutritional Laboratory Markers in Malnutrition. *J Clin Med*. 2019 May 31;8(6):775. doi: 10.3390/jcm8060775. PMID: 31159248; PMCID: PMC6616535
 - 12) Seltzer MH, Bastidas JA, Cooper DM, Engler P, Slocum B, Fletcher HS. Instant Nutritional Assessment. *J Parenter Enter Nutr*. 1979;3(3):157-9
 - 13) Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O'Reilly DS, Foulis AK, Horgan PG, McMillan DC. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer*. 2011

Nov;47(17):2633-41. doi: 10.1016/j.ejca.2011.03.028. Epub 2011 Jul 1. PMID: 21724383.

- 14) Médica GA, Aula G, Madrid M, Durán Alert P, Milà Villarroel R, Formiga F, et al. Assessing risk screening methods of malnutrition in geriatric patients; Mini Nutritional Assessment (MNA) versus Geriatric Nutritional Risk Index (GNRI). *Nutr Hosp*. 2012;27(2):590–8
- 15) Zhang Z, Pereira SL, Luo M, Matheson EM. Evaluation of Blood Biomarkers Associated with Risk of Malnutrition in Older Adults: A Systematic Review and Meta-Analysis. *Nutr* 2017 Vol 9 Page 829. 2017 Aug;9(8):829
- 16) Loftus TJ, Brown MP, Slish JH, Rosenthal MD. Serum Levels of Prealbumin and Albumin for Preoperative Risk Stratification. *Nutr Clin Pract Off Publ Am Soc Parenter Enter Nutr*. 2019 Jun;34(3):340–8
- 17) Norman K, Pichard C, Lochs H, Pirlich M. Prognostic impact of disease-related malnutrition. *Clin Nutr Edinb Scotl*. 2008 Feb;27(1):5–15
- 18) Banh L. Serum proteins as markers of nutrition: What are we treating? *Pract Gastroenterol*. 2006 Oct 1;30:46–64
- 19) Sieske L, Janssen G, Babel N, Westhoff TH, Wirth R, Pourhassan M. Inflammation, Appetite and Food Intake in Older Hospitalized Patients. *Nutr* 2019 Vol 11 Page 1986. 2019 Aug;11(9):1986
- 20) Forte WC, Gonzales CC, Carignani S, Mimica I. Avaliação de neutrófilos na desnutrição moderada [Evaluation of polymorphonuclear neutrophils in moderate malnutrition]. *Rev Assoc Med Bras* (1992). 1999 Apr-Jun;45(2):147-51. Portuguese.

doi: 10.1590/s0104-42301999000200010. PMID: 10413918

- 21) Tseng SH, Lee WJ, Peng LN, Lin MH, Chen LK. Associations between hemoglobin levels and sarcopenia and its components: Results from the I-Lan longitudinal study. *Exp Gerontol*. 2021 Jul 15;150:111379
- 22) Donini LM, Neri B, De Chiara S, Poggiogalle E, Muscaritoli M. Nutritional Care in a Nursing Home in Italy. *PLOS ONE*. 2013 Feb;8(2):e55804
- 23) Tsai MT, Tseng WC, Ou SM, Lee KH, Yang CY, Tarng DC, et al. Comparison of Simplified Creatinine Index and Systemic Inflammatory Markers for Nutritional Evaluation of Hemodialysis Patients. *Nutr* 2021 Vol 13 Page 1870. 2021 May;13(6):1870
- 24) Aggarwal HK, Jain D, Chauda R, Bhatia S, Sehgal R. Assessment of Malnutrition Inflammation Score in Different Stages of Chronic Kidney Disease. *PRILOZI*. 2018 Dec 1;39(2–3):51–61
- 25) Verma GK, Yadv RK, Chand R, Khan IA, Katiyar SBS, Singh M. Prognostic Significance of Serum Biochemistry Profile in Children With Severe Acute Malnutrition. *Cureus*. 14(11):e31266
- 26) Dent E, Wright ORL, Woo J, Hoogendijk EO. Malnutrition in older adults. *Lancet*. 2023 Mar 18;401(10380):951-966. doi: 10.1016/S0140-6736(22)02612-5. Epub 2023 Jan 27. PMID: 36716756
- 27) Ho KM, Morgan DJ, Johnstone M, Edibam C. Biological age is superior to chronological age in predicting hospital mortality of the critically ill. *Intern Emerg Med*. 2023 Oct;18(7):2019-2028. doi: 10.1007/s11739-023-03397-3. Epub 2023 Aug 28. PMID: 37635161; PMCID: PMC10543822

- 28) Rutledge, J., Oh, H. & Wyss-Coray, T. Measuring biological age using omics data. *Nat Rev Genet* 23, 715–727 (2022)
- 29) Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L, Horvath S. An epigenetic biomarker of ageing for lifespan and healthspan. *Ageing (Albany NY)*. 2018 Apr 18;10(4):573-591. doi: 10.18632/aging.101414. PMID: 29676998; PMCID: PMC5940111
- 30) Galetta F, Carpi A, Abraham N, Guidotti E, Russo MA, Camici M, Antonelli A, Franzoni F, Santoro G. Age related cardiovascular dysfunction and effects of physical activity. *Front Biosci (Elite Ed)*. 2012 Jun 1;4(7):2617-37. doi: 10.2741/e570. PMID: 22652665; PMCID: PMC5673121
- 31) Laconi, E., Marongiu, F. & DeGregori, J. Cancer as a disease of old age: changing mutational and microenvironmental landscapes. *Br J Cancer* 122, 943–952 (2020).
<https://doi.org/10.1038/s41416-019-0721-1>
- 32) Liu JP. Molecular mechanisms of ageing and related diseases. *Clin Exp Pharmacol Physiol*. 2014 Jul;41(7):445-58. doi: 10.1111/1440-1681.12247. PMID: 24798238
- 33) Bocklandt S, Lin W, Sehl ME, Sánchez FJ, Sinsheimer JS, Horvath S, Vilain E. Epigenetic predictor of age. *PLoS One*. 2011;6(6):e14821. doi: 10.1371/journal.pone.0014821. Epub 2011 Jun 22. PMID: 21731603; PMCID: PMC3120753
- 34) Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018 Jun;19(6):371-384. doi: 10.1038/s41576-018-0004-3. PMID:

29643443

- 35) Zhang W, Qu J, Liu GH, Belmonte JCI. The ageing epigenome and its rejuvenation. *Nat Rev Mol Cell Biol.* 2020 Mar;21(3):137-150. doi: 10.1038/s41580-019-0204-5. Epub 2020 Feb 4. PMID: 32020082

