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Differences in levels of albumin, ALT, AST, γ -GT and creatinine in frail, moderately healthy and healthy elderly individuals

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Abstract

Background: Reference intervals are widely used as decision tools, providing the physician with information about whether the analyte values indicate ongoing disease process. Reference intervals are generally based on individuals without diagnosed diseases or use of medication, which often excludes elderly. The aim of the study was to assess levels of albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and γ -glutamyl transferase (γ -GT) in frail, moderately healthy and healthy elderly indivuduals.

Methods: Blood samples were collected from individuals >80 years old, nursing home residents, in the Elderly in Linköping Screening Assessment and Nordic Reference Interval Project, a total of 569 individuals. They were divided into three cohorts: frail, moderately healthy and healthy, depending on cognitive and physical function. Albumin, ALT, AST, creatinine and γ -GT were analyzed using routine methods.

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Results: Linear regression predicted factors for 34% of the variance in albumin were activities of daily living (ADL), gender, stroke and cancer. ADLs, gender and weight explained 15% of changes in ALT. For AST levels, ADLs, cancer and analgesics explained 5% of changes. Kidney disease, gender, Mini Mental State Examination (MMSE) and chronic obstructive pulmonary disease explained 25% of the variation in creatinine levels and MMSE explained three per cent of γ -GT variation.

Conclusions: Because a group of people are at the same age, they should not be assessed the same way. To interpret results of laboratory tests in elderly is a complex task, where reference intervals are one part, but far from the only one, to take into consideration.

Keywords: aging; analyte; clinical interpretation; frail; reference interval.

Introduction

Reference intervals are widely used as decision support tools, providing the physician with information if the analyte values are indicating an ongoing disease process or not [1–3]. Although many patients seeking health care are in their eighties, reference intervals are generally based on young and middle-aged individuals, i.e. without diagnosed diseases or use of medication.

There are a few attempts to include elderly persons in the development of reference intervals, but persons aged 80 years or more are most often excluded from participating (because of age, chronic diseases and use of medications) and the proposed reference intervals do seldom represent persons aged 80 years or more [4–6]. For example, in the Nordic Reference Interval Project (NORIP) (n=2777; healthy individuals 18–90 years) [4], reference intervals were proposed and adopted as standards by clinical chemistry laboratories in the Nordic countries for 25 analytes. The number of individuals who were 80 years and older were 64.

Recently, reference intervals for 24 analytes in blood were established from approximately 12,000 apparently healthy individuals, aged 3–79 years, in the Canadian Health Measure Survey (CHMS) [5]. The CHMS also proposed different reference intervals according to gender and age, with cohorts younger than 80 years (3-5, 6-7, 8-19, 20-39, 40-59 and 60-79 years old). Furthermore, in a large Chinese study, more than 7000 individuals, aged 20-79, were examined to determine reference intervals for sodium, potassium and chloride [6]. Based on inclusion criteria, 4500 individuals (64%) were considered sufficiently healthy to serve as reference population. There are, however, a few exceptions with a focus on persons 80 years and older, e.g. Helmersson-Karlqvist recently studied an 80-year-old population (n = 531; patients with "known diagnosis of diabetes or fasting glucose values >7.0 mmol/L" were excluded) and calculated reference intervals for 34 frequently used laboratory tests [7].

Establishing reference intervals for an elderly population is, however, complicated as it is unclear if changes in levels of analytes are affected by aging per se or disease. The biological aging processes causes a general decline in organ functions and immune system, with loss of muscle mass, renal function decline, increased risk of heart disease, infections and stroke as some of the consequences [8-10].

There are those who suggest that low alanine aminotransferase (ALT) is associated with frailty and reduced survival [11] and that we should have specific reference intervals for ALT for older individuals [12]. On the other hand, some studies do not report any differences at all in ALT levels between younger and older individuals [13], and the same contradictory results have been shown for creatinine [4, 5]. For aspartate aminotransferase (AST), special reference intervals have been suggested for elderly individuals in both the Canadian and the Swedish study [5, 7], but NORIP suggests the same intervals for all individuals >18 years [4]. Nor does complete agreement exist for γ -glutamyl transferase (γ -GT). Both the Canadian study [5] and the NORIP [4] proposed higher reference intervals for the elderly compared with younger individuals.

However, "elderly individuals" make up a heterogeneous group ranging from individuals with diseases and impairment, in need of nursing care around the clock, to healthy individuals managing their daily life independently. A key question this raise is, do levels of analytes differ between sub cohorts of elderly based on frailty? This was addressed in a recent investigation in which we found that levels of some common analytes, in frail multidiseased elderly, i.e. nursing home residents (NHRs) aged ≥80 years, differed from levels in healthy reference populations of blood donors 18-60 years and the NORIP population of 18- to 90-year-olds. Frail elderly individuals

had lower levels of immunoglobulin M (IgM) and higher levels of IgG and complement components 3 and 4 (C3) and C4) compared with blood donors [14]. Lower levels of ALT, albumin, phosphate and sodium and higher levels of creatinine and urea were seen in the frail compared with the NORIP reference population (data not shown). No differences were found for IgA, AST, γ-GT or lactate dehydrogenase between NHRs and NORIP subjects. However, it is not known whether these findings were related to morbidity, in terms of chronic disease, cognitive and physical impairment, or just to the aging process. Therefore, we found it valuable to further evaluate analytes in relation to morbidity in combination with cognitive and physical ability.

The aim of the present study was to assess levels of albumin, ALT, AST, creatinine and γ -GT in relation to physical and cognitive conditions in three different cohorts of elderly individuals, defined as frail, moderately healthy and healthy.

Materials and methods

Data originated from three different projects for which blood samples had been collected from individuals ≥80 years: NHRs [15], a cohort in the Elderly in Linköping Screening Assessment (ELSA 85) [16] and NORIP [4].

NHR study

Regarding NHRs, data on 168 elderly individuals living in nursing homes, mean age 88.0 year (80-101 year), 75% women, were collected during 2007-2009 [15]. Data on chronic diseases, medications and functional and cognitive status were obtained from the medical records and using validated assessment tools, i.e. the Katz Index of Independence in Activities of Daily Living (Katz-ADL) for personal activities of daily living (PADLs), the ADL staircase with instrumental activities of daily living (IADLs) and the Mini Mental State Examination (MMSE) [17-19]. There are six categories of PADLs: bathing, dressing, toileting, transfer, continence and feeding, and four categories of IADLs: cooking, transportation, shopping and cleaning. The total ADL score ranges from 0 to 10, where 0 = independency in all variables and 10 = dependency in all variables [17, 18]. The MMSE consists of 21 questions testing memory, naming, orientation, attention and constructive ability [19]. The maximum score is 30 and a score of <27 indicates cognitive impairment.

In the morning, non-fasting venous blood samples were collected in evacuated tubes, using ethylenediamine tetra-acetic acid (EDTA) as anticoagulant, and centrifuged. The plasma was transferred into secondary tubes and frozen to -80 °C until analyzed in 2015 using an automated analyzer (Siemens ADVIA 1800 Siemens Healthcare Diagnostics. Inc., Japan/Canada). Methods used were albumin bromcresol purple (BCP), enzymatic creatinine and the IFCC reference measurement procedure at 37 °C for ALT, AST and γ-GT.

ELSA study

In the ELSA 85 cohort, data on 338 elderly individuals aged 85 years, 57% women, were collected during 2007–2008 [16]. Data on chronic diseases, medications and functional and cognitive status based on PADLs, IADLs [20] and MMSE scores [19] were collected from the medical records and questionnaires administered at a reception visit. Assessment of PADLs categorized as "manage without assistance", "need some assistance" and "need a lot of assistance". Instrumental ADLs were assessed based on the Instrumental Activities Measure (IAM), which tests the ability to manage locomotion outdoors, prepare a simple meal, cook, use public transportation, do smallscale shopping, do large-scale shopping, clean and wash, with ability levels of "no difficulty", "some difficulties", "difficult" and "too difficult" [20]. Venous blood samples were collected in tubes, using lithium heparin as anticoagulant, and centrifuged. The plasma was transferred into secondary tubes and kept at -80 °C until analyzed with automated analyzer, for albumin (BCP) and creatinine Jaffé with Siemens ADVIA 1800 (Siemens Healthcare Diagnostics. Inc., Japan/ Canada) and ALT, AST, γ -GT (IFCC 37 °C) with Selectra Pro M (ELITech Group, Puteaux, France).

NORIP study

From the NORIP 2.3% of the total reference population, 63 individuals 80 years and older were included, mean age 81.9 years (80-90 years), and 51% were women [4]. Inclusion criteria were as follows: subjectively healthy; not admitted to hospital or seriously ill during the past month; not consuming >24 g pure alcohol during the last 24 h; having taken no prescribed drugs during the past 2 weeks; and no smoking during the hour before blood sampling. In the NORIP neither MMSE scores nor ADLs were measured, but the inclusion criteria neither excluded cognitively nor physically impaired persons. Venous blood was collected during 2000 and 2001, in plain tubes for serum and tubes using lithium heparin as anticoagulant, and centrifuged. Some of the samples were collected for analysis at the laboratory and some to a central bank. The plasma and the serum transferred into secondary tubes and frozen at the participating laboratories at -80 °C until analyzed. For enzymes, only Vitros and IFCC 37 °C were accepted for used. Each laboratory received five controls with instructions on how to handle comparisons between the different labs possible.

Study cohorts: frail, moderately healthy and healthy individuals

A secondary variable was created, dividing the individuals into three cohorts: frail, moderately healthy and healthy. A frail NHR in the present study was defined as a person with a Katz-ADL score of ≥5 points [18, 19] and an MMSE score of 0-26 [21]. For ELSA 85, "frailty" was classified as all eight IAM items being assessed "difficult" or "too difficult" and needing some kind of assistance to manage any of the following: bathing, dressing, toileting and feeding and having an MMSE score of 0-26. Individuals classified as moderately healthy had an ADL score equivalent of ≥5 points, or an MMSE score of 0-26 or some kind of chronic disease or diseases. Healthy elderly people were classified as individuals with ADL score equivalent of ≤4 and an MMSE score of 27-30 and without any chronic disease.

Because different anticoagulants were used in the different study cohorts, i.e. EDTA for NHRs and lithium heparin for the ELSA 85 and NORIP cohorts, analytes studied for this investigation were dependent on being suitable for EDTA. Hence, albumin, ALT, AST, creatinine and γ-GT were analyzed using routine methods, in Swedish Board for Accreditation and Conformity Assessment-accredited laboratories based on ISO/IEC 17025 standards.

Statistics

Descriptive statistics were constructed using medians and 25th to 75th percentiles and presented in box plots with whiskers indicating minimums and maximums. Pearson's and Spearman's correlations were used to determine correlations between analytes and independent variables, namely, age, gender, height, weight, smoking status; dementia, asthma, presence of chronic obstructive pulmonary disease (COPD), stroke and diabetes, as well as cancer, heart, liver, kidney, autoimmune and/or thyroid disease; use of medication such as sedatives, antidepressants, analgesics and sleeping pills; and scores regarding ADLs and the MMSE (Table 1). Analysis of variance (ANOVA) and Tukey's post hoc test were used to compare mean differences between the different cohorts. The following analytes were considered as dependent variables in the regression analysis: albumin, ALT, AST, creatinine and γ-GT. Linear regression models were developed to explore variables affecting changes in levels of analytes. Variance inflation factors between independent variables were assessed and no variance >1.3 occurred. For statistical calculation, we used PASW Statistics 24 (SPSS Inc., Chicago, IL, USA).

Table 1: Distribution of chronic disease and use of medications in 569 elderly persons, divided into frail (n = 152), moderately healthy (n=254) and healthy (n=163) individuals.

Condition	Frail individuals n (%)	Moderately healthy individuals n (%)	Healthy individuals n (%)
Dementia	101 (66.4)	16 (6.3)	0 (0)
Chronic heart disease	87 (57.2)	73 (28.7)	0 (0)
Asthma	7 (4.7)	24 (9.3)	0 (0)
COPDa	7 (4.7)	15 (5.8)	0 (0)
Stroke	53 (34.9)	56 (22)	0 (0)
Diabetes mellitus	30 (19.7)	63 (24.8)	0 (0)
Chronic liver disease	1 (0.7)	3 (1.2)	0 (0)
Chronic kidney disease	8 (5.4)	5 (1.9)	0 (0)
Cancer	36 (23.7)	50 (19.7)	0 (0)
Autoimmune disease	19 (12.8)	19 (7.4)	0 (0)
Thyroid disease	16 (10.7)	29 (11.3)	0 (0)
Medication: sedatives	53 (35.6)	25 (9.7)	5 (3.1)
Antidepressants	81 (53.3)	36 (14.2)	9 (5.5)
Analgesics	83 (54.6)	155 (61)	55 (33.7)
Sleeping pills	39 (26.2)	48 (18.7)	17 (10.4)

^aChronic obstructive pulmonary disease.

Ethics

All participants, or their next of kin, gave written informed consent. It was made clear that participation was voluntary and could be withdrawn at any time. The Local Ethics Committee of the Faculty of Health Sciences, Linköping University (Dnr: M-8206 and Dnr: 141-06), and the health service directors of community care and geriatric staff nurses gave their permission to conduct the study.

Results

As expected, frailty was most common among NHRs but was also present in the ELSA 85 cohort. No one in the NORIP cohort was classified as moderately healthy or frail (Table 1). In our frail cohort (n=152), the mean age was 88.0 years, SD 4.5 (range 80–101 years); 73.7% were women. The number of moderately healthy individuals was 254, mean age 85.2 years, SD 1.37 (range 81–94 years); 59.8% were women. In the healthy cohort (n=163), the mean age was 83.8 years, SD 2.08 (range 80–90 years), and 54.0% were women.

Distributions of albumin, ALT, AST, creatinine and γ-GT are presented in box plots, stratified by gender and into frail, moderately healthy and healthy (Figure 1A-E). For albumin, lower levels were found in frail compared with moderately healthy and healthy individuals (p < 0.01). When investigating men and women separately, the same pattern was seen (p < 0.01). For ALT, frail individuals had lower levels compared with moderately healthy and healthy individuals (p<0.01). When investigating men and women separately, we found the same pattern (p < 0.01). Lower levels for AST occurred in moderately healthy individuals compared with frail individuals (p < 0.01). For women, the moderately healthy group had lower levels compared with the healthy group and the frail group (p<0.05), but for men, there were no differences between the cohorts. Moderately healthy individuals had higher levels of creatinine compared with the other groups (p < 0.01). Among women, moderately healthy women had higher levels compared with both frail and healthy women (p < 0.01). Moderately healthy men had higher levels compared with healthy men (p<0.05). Levels of γ -GT were higher for frail men and women together (p<0.01) and for women separately (p < 0.05), compared with healthy and moderately healthy individuals. When excluding the two highest values, frail individuals still had significantly higher levels compared with healthy and moderately healthy individuals (p < 0.05 compared with p < 0.01before exclusion).

Pearson's and Spearman's analysis, preceding the linear regression, showed several correlations between background variables (Supplemental Table 1). Predicting factors for changes in albumin were ADLs, gender, stroke and cancer, explaining 34% of the variance (Supplemental Table 2A). Scores for ADLs, gender and weight explained 15% of changes in ALT (Supplemental Table 2B). For AST levels, ADLs, cancer and analgesics explained 5% of changes (Supplemental Table 2C). Kidney disease, gender, MMSE and COPD explained 25% of the variation in creatinine levels (Supplemental Table 2D). Three percent of the variation in γ -GT levels was explained by MMSE.

Predictive factors for changes in albumin in the frail cohort were thyroid disease and having had stroke, which explained 11% of variance (Supplemental Table 2A). Age and weight correlated significantly with ALT (n=137), while one variable that correlated significantly with AST (n=133) in the frail cohort was diabetes. No regressions were performed in the levels in the frail. Kidney disease, gender, cognitive status and asthma explained about 34% of the variation in creatinine levels (Supplemental Table 2D). The variation in γ -GT levels in the frail cohort was explained by use of analgesics in 3.4% (n=136).

Predicting factors for changes in albumin in the moderately healthy cohort were age, gender and ADLs, explaining 11% of the variance (Supplemental Table 2A). Height and age explained 8% of changes in ALT (Supplemental Table B). For AST levels, cancer and analgesics explained 6% of changes (Supplemental Table 2C). Gender and kidney disease explained 17% of the variation in creatinine levels (Supplemental Table 2D). One variable, heart disease, correlated significantly with γ -GT (n=234) in the moderately healthy cohort. No regression was performed.

Correlations between analytes in the healthy elderly (n=163) and the independent variables age, gender, height, weight, smoker, sedatives, antidepressants, analgesics and sleeping pills were examined. None of the independent variables correlated significantly with albumin in the healthy cohort. Age together with antidepressant use explained 10% of changes in ALT (Supplemental Table 2B). About 8% of the variation in AST levels was due to age (n=144). The variation in creatinine levels in the healthy cohort was explained by gender in 14.6% (n=162). One variable, analgesics, correlated significantly with γ -GT (n=122) in the healthy cohort. No regression was performed.

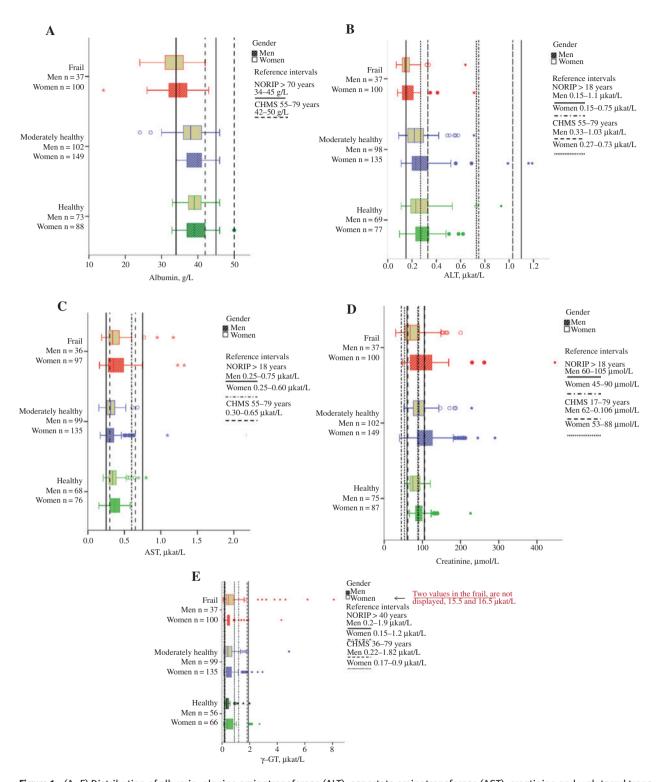


Figure 1: (A–E) Distribution of albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and γ -glutamyl transferase (γ -GT) levels across the three sub cohorts, of frail (n=152), moderately healthy (n=254) and healthy (n=163) elderly individuals. Medians and variation between 25th and 75th percentiles are presented in the box, and whiskers indicate minimum and maximum values. Circles represent values between 1.5 and three times the interquartile range, and asterisks (*) represent values that are more than three times the interquartile range. (For ALT, AST and γ -GT 1 U/L = 0.017 μ kat/L.)

Discussion

The World Health Organization predicts that the proportion of people aged 60 years and older will increase from 11% to 22% between 2000 and 2050 [22]. They also predict that individuals aged 80 years or more will almost quadruple between 2000 and 2050, resulting in 395 million elderly individuals, probably with need for extensive nursing and medical care. In Sweden, the number of elderly people in senior living has risen from 11,000 to 33,000 between 2000 and 2008 [15].

Reference intervals for laboratory analytes are useful for the physician when investigating individuals to monitor the course of, or detect, disease. The whole idea of using reference intervals is to be able to make comparisons with healthy individuals in order to find out what makes a person not feel good and to detect diseases. It becomes a problem when you want to find out if a person with one or more diseases may have another disease, which is common in the case of older individuals. In the present study, we have examined levels of five commonly used analytes in individuals with different diseases and in relation to physical and cognitive conditions in three different cohorts of elderly people, defined as frail, moderately healthy and healthy.

The lower levels of albumin in frail men and women, both together and separately by gender, were in line with previous results when we studied individuals in NHRs, who were all frail, compared with albumin levels in the NORIP reference population [14]. The low level of albumin in frail elderly individuals is likely due to malnutrition and ongoing inflammatory processes [23]. Differences to reference intervals proposed by CHMS and NORIP are that the age differs between the populations and that ethnicity may be a contributing factor. It is also conceivable that differences have arisen because of the different analytical methods used. In the CHMS, a standardized dry chemical method using Ortho Vitros 5600 FS was applied, compared with different standardized methods used in NORIP. Frail men and women had lower levels of ALT compared with moderately healthy and healthy men and women (p < 0.01), which is in line with previous results [15, 18]. The reason for this is uncertain, but the finding may be due to declining function of the liver with increasing age. Both NORIP and the CHMS suggest higher reference intervals than the levels we saw in our population, which can be misleading and may indicate risk for missing diseases in the elderly.

For AST, moderately healthy individuals had the lowest levels compared to the frail (p<0.01). In our previous study, no differences were found between frail and healthy elderly people [14]. In the present study, AST in

moderately healthy women had lower values compared with both healthy and frail elderly women (p < 0.05), but no difference between the cohorts was detected for men.

Previously, we had found that frail elderly individuals had higher levels of creatinine compared with the healthy elderly [14]. In the present study, moderately healthy men and women together, and women alone, had higher levels of creatinine than healthy and frail individuals (p<0.01). Moderately healthy men had higher creatinine levels compared with healthy men (p < 0.05). A cause could be that the kidney function begins to decline in individuals who are moderately healthy and decreased muscle mass can be the reason in the frail.

In the present study, frail elderly individuals had higher levels of γ -GT compared to healthy and moderately healthy individuals, which we had not found in our previous study [14]. Although the men did not show any significant differences between the cohorts, for women, the frail cohort had higher levels compared with both the healthy and the moderately healthy women (p < 0.05). After exclusion of the two highest γ-GT levels, the frail cohort still had higher levels compared with the moderately healthy and healthy cohorts (p < 0.05).

Investigating the levels of analytes in the elderly population is complex, mostly because of chronic diseases, comorbidity and medication. Others [6-8] have tried to develop reference intervals and have then used the guidelines that provide a template to establish the standard interval. The standardization requires that no individuals with disease and using medication are included in the reference population. It should be noted that the group of older people has increased greatly since these guidelines were created.

A limitation of the present study is that blood from the participating individuals was collected from three different studies and the selection of anticoagulants differed. Thus, in the study of NHRs [12], EDTA was used as anticoagulant, whereas in the ELSA 85 [19] and NORIP [4] cohorts, addition of lithium heparin was used. However, in the present study, the EDTA anticoagulant limited us in the choice and the numbers of investigative analytes.

One strength of this study is that we included a large population of elderly people, a total of 569 individuals aged ≥80 years. Their different health status enabled us to divide them into three cohorts, those who were frail, those who were healthy and those who fell into a category between the others, the moderately healthy. The middle cohort, with the largest number, 254 (45%), consisted of individuals who also probably sought primary health care most often. The frail elderly often live in special accommodation and are taken care of by trained staff, whereas the healthy do not need to seek medical care. Dividing the population according to health status could be seen as a weakness of the present study, but it was one way of studying this complex field, and illustrating the reality really well. Had we included only individuals without diseases and medicaments, as is commonly done, the results would have been applicable to very few elderly.

The present study shows that just because a group of people are at the same age, they should not automatically be assessed in the same way. Most of the people over 80 years have one or more chronic diseases, and many of them are on medications. In addition, these diseases and medications could have debuted at different ages and thus impacted the body different lengths of time. To interpret results of laboratory tests in elderly is a complex task, where current reference intervals are one part, but far from the only one, to take into consideration. The present study presents findings in relation to common analytes. The study emphasizes the need for finding solutions in an aging population. We need to focus on how to deal with the challenge that we and most countries are facing.

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