Clinical use of conventional reference intervals in the frail elderly

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Introduction

Measurement of biomarkers in plasma is essential when physicians assess the presence of disease, and make treatment decisions (1). This is of special importance in frail elderly individuals, as the presence of nonspecific signs and symptoms and lack of specific ones are common for example in relation to infection (2). Biomarkers play a crucial role when physicians confirm or refute the presence of disease. However, actual reference values for biomarkers are mainly based on young, healthy individuals, excluding both elderly and frail individuals.

The finding of altered concentrations of a biomarker, may be achieved by comparing with previously observed values, or with a set of appropriate group-based reference values [8, 9]. Common criteria when making reference values for blood samples is to ask if the person is healthy [8] and to exclude individuals with disease or receiving drugs [10, 11]. Healthy people, such as military personnel, hospital staff or blood donors 18 - 65 years of age, are frequently used to represent the reference population . The group-based reference values are often condensed into a reference interval defined by two reference limits [13]. The procedures to obtain reference intervals may differ from simple intuitive estimation of the available data to complex statistical techniques. A frequently used convention is that the reference interval should contain the central 0.95 fraction (95 %) of the reference distribution. To use standard methods calculating mean $\pm$ SD, if the values are normally distributed, is another way However, other fractions or an asymmetric location of the reference interval may be more appropriate in particular cases [13].. For values that are not normally distributed, the square root or log transformation can be used [12, 14]. The International Federation of Clinical Chemistry (IFCC) and the Scandinavian Committee on Reference Values have approved strict rules for generating reference values [15-17].

The ageing process is influenced by a variety of defence functions or anti-stress responses activated by stressors including physical (UV and gamma-radiation), chemical (oxygen-free radicals and reducing glucose) and biological (viruses, bacteria) agents [18]. Age is accompanied by a general decline in organ functions and loss of muscle mass as a result of malnutrition and meno/andropause, Chronic heart failure and stroke are consequences of age-related alterations in cardiac and vascular structure and function [20]. Also renal function declines with ageing, mainly due to changes in the glomeruli, but also because of diabetes, heart failure, malignancy and inflammatory systemic diseases [20]. Sensory losses, chewing and swallowing problems reduce dietary intake, which can lead to malnutrition [21]. Hence, ageing is associated with increased risk of morbidity, hospital admissions and dependency in daily care [22].

The relative number of apoptotic cells increase in people 80 years and older [23] and the functions of the immune system deteriorate [24]. An age-related decrease in the amount of peripheral blood T cells and a slower rise and earlier decline of the antibody response occurs [24]. Ageing of the immune system plays a role in the increased susceptibility to acute infections [2, 24], such as influenza, pneumonia, urinary tract infection and intra-abdominal sepsis [25]. Furthermore, the presence of low-grade inflammation is proposed to cause not only ageing itself, but also age-related diseases, such as Alzheimer's disease, myocardial infarction, stroke and diabetes type 2 [26, 27]. Taken together, the compilation of reference intervals for the elderly is complicated by a number of factors, including the presence of multi-system disease, the effects of diet and malnutrition, the use of medication and the physiologic and anatomic changes associated with age [11].

To our knowledge only a few prior attempts have been made to establish reference intervals for biomarkers in elderly individuals and different approaches have been used. Bohnen et al. recruited subjects by advertising in newspapers and measured biomarkers in healthy individuals divided into four age groups from 20 to 80 years with 20 persons in each group [1]. The inclusion criteria were no chronic serious illness and no acute medical condition in the past three months, resulting in only five persons in the age ≥75 years. The results showed higher levels for individuals over 75 years of age compared to the younger for total cholesterol, triglycerides, aspartate aminotransferase (AST), creatinine, gamma-glutamyltransferase (γ-GT) and urea and lower for albumin. Bäck et al. included individuals 20 – 80+ years, and proposed reference intervals for common biomarkers and immunoglobulins[12]. In a study by Kallner et al. [28], patients aged from childhood to 98 years old, visiting the outpatient clinics during 18 consecutive months, were included to make reference intervals related to age, sex and if healthy or not. Individuals visiting only once were referred to as "non-diseased". The results showed increased levels of alanine aminotransferase (ALT) and γ-GT and a slowly decrease in albumin concentrations with age [28]. For Lock et al. the purpose was to investigate reference intervals for immunoglobulins of elderly individuals, resulting in about 500 over 70 years old [29]. No differences in levels of IgA, but decreased levels of IgG and IgM in elderly persons were found compared to the younger. Same-sex twin pairs aged 80+ were included in a study by Nilsson et al. [30]. Six years later, 261 of the 535 included were still alive, and thus defined as healthy. High levels for urea, urate, γ-GT, free thyroxin and homocystein all correlated with mortality. In females, low plasma values for albumin and total cholesterol were associated with increased mortality [30].

Huber et al. studied biomarkers reference intervals for 75-year-old individuals and used extensive clinical, neurological, biochemical, psychological, genetic and radiological analyses to select apparently healthy individuals [31]. Of the 120 included individuals only 27 participants were completely free from disease. Despite this, Huber et al. suggested reference intervals for 75-year-old apparently healthy individuals in clinical chemistry. None of above attempts has as yet resulted in the routine use of specific reference intervals for the elderly for the most common biomarkers.

The aim of the present study was to establish whether current reference intervals for immune parameters (immunoglobulin A (IgA), IgG, IgM, complement factor 3 (C3), C4) and chemical biomarkers (ALT, albumin, AST, creatinine, γ-GT, lactate dehydrogenase (LDH), Na, phosphate and urea) are valid for older frail individuals.

Materials and methods

Study population

Individuals included in another study[25], 237 nursing home residents (NHR), were invited to participate in the present study. Of them 138 were 80 years and older, in the text called NHR, gave informed consent for blood sampling and were included in the present study. All of them needed daily care and support, assessed with the ADL (activities of daily living) Staircase, based on Katz ADL index {Takata, 2013 #23} (32). They all lived in special housing for elderly. Mean age was 86.8 years, 66% were women and about 22% had multiple disease conditions. Chronic obstructive pulmonary disease (COPD) was more frequent in men and dependency in ADL higher in women ($P < 0.05$ respectively) (Table 1). Almost half of the NHR received paracetamol $\geq$ 3 g /day and 15% was malnourished [33]. Only nine (7%) of 138, although aged, were assessed as "healthy", in terms of free from heart disease, autoimmune disease, dementia, stroke, diabetes mellitus type 2, malnutrition or receiving paracetamol. All the included (n= 138) belong to frail elderly, as defined by Ernsth-Bravell et. al.[34].

Venous blood was collected in EDTA tubes, in the morning 8-11 a.m., centrifuged, and frozen at -70°C until analysed. Analytes studied were dependent on being suitable for EDTA. IgA, IgG, IgM, C3, C4, ALT, albumin, AST, creatinine, γ-GT, LDH, phosphate, sodium and urea were analysed with accredited routine methods at Ryhovs Hospital, County Council of Jönköping. One IgA value from NHR and two IgM values have been truncated, because of their extremely different values, but for chemical biomarkers all available NHR data have been used.

Table 1

Reference populations

Blood donors

The samples originated from two Swedish blood donor populations at the University Hospital in Linköping. The general criteria for blood donors in Sweden are healthy individuals at the age of 18 – 64 years. The values for C3 and C4 were based on 123 blood donors, 22 – 63 years, mean 41.0 and 19.5% were women. For the donors used (n=189) for analysis of IgA, IgG and IgM age and gender were unknown, but it was probably about the same. As for NHR were collected venous blood was collected in EDTA tubes which are then centrifuged and frozen at -70°C until analysed. Plasma was analysed by accredited routine methods at the University Hospital in Linköping. The Beckman Immage Nephelometry Index (Beckman Instruments Inc, USA) was used for measurement of immunology parameters. From blood donors all available data were used for statistical analysis. The reference intervals for IgA, IgG and IgM have later been adjusted. Current reference intervals for immune parameters are presented in Table 2.

Table 2

The Nordic reference interval project (NORIP)

From the NORIP project the total database has been used with original data, i.e. NORIP raw origin. The total population included 2777 individuals, 18 – 90 years, mean 46.6 and 53% were women [35]. Since only 64 individuals (2.3%) 80 years and older were included, i.e. NORIP raw origin 80, comparisons with NHR have been done both with NORIP raw origin and NORIP raw origin 80. Inclusion criteria were subjectively healthy, $\geq$ 18 years, not pregnant or breastfeeding, not submitted to hospital nor seriously ill during the past month, not consuming > 24 g pure alcohol during the last 24 h, no prescribed drugs other than oral contraceptives or oestrogen during the past 2 weeks, no smoking during the hour before blood sampling. Venous blood was collected in the morning, centrifuged, and frozen at -80°C until analysed with routine methods at the 102 participating laboratories [35]. The samples were analyzed intermingled with internal quality control samples in order to minimize between-laboratories bias. For statistical comparison all available data from NORIP were used. In Table 3 reference intervals proposed by NORIP are presented.

Table 3

Statistics

Student´s *t*-test was used to compare values between NHR, the blood donors, NORIP raw origin and NORIP raw origin 80 and also to compare subgroups within NHR. The statistical significance was defined as $p < 0.05$ and PASW Statistics 20 software (SPSS Inc, Illinois, USA) was used. In the box plots median and the 25:th and 75:th percentiles are presented.

Ethics

All the participants or the next of kin gave both oral and written informed consent. The Ethics Committee of the Faculty of Health Sciences, Linköping University (Dnr: 99017) and the health service directors of community care approved the study.

Results

Comparison between NHR and reference populations

No differences in mean levels of IgA were found between NHR and blood donors. IgG mean was higher in NHR, 11.4 g/L compared to blood donors, 10.7 g/L ($p<0.05$, Figure 1a). For IgM a lower mean level was found in NHR, 1.00g/L compared to blood donors, 1.75 g/L, ($p<0.001$, Figure 1b). Both C3 and C4 mean levels were higher in NHR, 1.42 g/L and 0.38 g/L, respectively, compared to the blood donors, 0.66 g/L and 0.23 g/L ($p<0.001$, Figure 1c and d).

Figure 1

The distribution of AST, γ-GT and LDH did not show any differences in mean levels between NHR and NORIP raw origin or NORIP raw origin 80.

For NHR a lower mean level was found for ALT, 0.15 µkat/L compared to NORIP raw origin, 0.40 µkat/L or NORIP raw origin 80, 0.32 µkat/L ($p<0.001$, Figure 2a). Albumin mean was lower for NHR, 37 g/L then NORIP raw origin, 41 g/L or NORIP raw origin 80, 39 g/L($p<0.05$, Figure 2b). For NHR mean sodium showed lower values, 139 mmol/L then NORIP raw origin, 141 mmol/L or NORIP raw origin 80, 141 mmol/L ($p<0.01$, Figure 2c).

Mean phosphate for NHR was 1.0 mmol/L and for NORIP raw origin and NORIP raw origin 80 1.1 mmol/L. Difference occurred between NHR and NORIP raw origin ($p<0.001$, Figure 2d).

For NHR creatinine mean was higher, 108 µmol/L then NORIP raw origin, 73 µmol/L or NORIP raw origin 80, 80 µmol/L ($p<0.001$, Figure 2e). Urea was higher with mean for NHR, 8.9 mmol/L compared to NORIP raw origin, 5.0 mmol/L or NORIP raw origin 80, 6.2 mmol/L ($p<0.001$, Figure 2f).

Figure 2

Comparison between subgroups within the NHR

The distribution of IgA, AST**,** γ-GT, LDH, Na and Phosphate did not show any differences in mean levels between NHR with or without medication with antidepressants or paracetamol, malnutrition, physical and cognitive status or the presence of heart disease, autoimmune disease or COPD (Table 4).

Significant decreased IgG was found for NHR with daily medication with paracetamol, while IgM was decreased in association with malnutrition, autoimmune disease and COPD. Significant higher C3 and C4 occurred in NHR with daily medication with paracetamol and at ADL $\geq$ 5. Albumin was significantly lower in NHR using antidepressants, with malnutrition, dementia or ADL > 5. ALT levels were significantly higher in case of daily paracetamol treatment. Increased levels of urea and creatinine occurred in the presence of heart disease, while urea was decreased in the presence of autoimmune disease.

The nine NHR individuals that were free from disease (heart or autoimmune disease, dementia, stroke, diabetes mellitus type 2, malnutrition) and did not receive paracetamol, showed no differences in levels of biomarkers, compared with the rest of NHR.

Table 4

Discussion

The results clearly show that there are significant differences in levels of some of the investigated chemical biomarkers in the NHR, compared to the current reference intervals. This could result in misleading or even dangerous assessments, since normal conditions may appear pathological, or the contrary, and thus lead to unnecessary treatment. As an example, an increase in IgG, IgM, C3 and C4 in frail elderly indicate an activated immune system, which may be a part of a chronic low-grade inflammation [26, 27]. As it is known that levels of interleukin 1 and 6 increase with ageing, and they in turn induce acute phase protein, these results seem reasonable [27]. In the present results, paracetamol was related to lower IgG levels in the NHR, which may not be discovered. However, our results are consistent with Lock [29],

who reported decreased levels of IgM in the elderly [29]. Another example of potential misinterpretations is that higher C3/C4 levels in the elderly could mask a consumption of complement as in glomerulonephritis. It seems like the most differences between NHR and the reference population for chemical biomarkers depends on the existence of disease. Differences in mean for ALT, albumin, creatinine, urea and sodium occurred between NHR and NORIP raw origin as well as between NHR and NORIP raw origin 80. For phosphate difference in mean occurred between NHR compared to NORIP raw origin, but not compared to NORIP raw origin 80. Levels of phosphate, however, seem to be affected by different conditions, even if no specific disease gave rise to significant differences (Table 4). As liver disease cause a rise in plasma of ALT, such condition might be missed in frail elderly individual. Furthermore in NHR using paracetamol every day, an overdose of paracetamol or high alcoholic consumption may not be discovered, as the ALT seems to be within current reference interval. In line with other studies [28, 31], we also noted that NHR individuals had deceased phosphate levels compared with younger individuals. Kidney insufficiency in elderly individuals with diabetes might not be discovered, as a high level of phosphate in plasma is interpreted as within current reference values. This is also significant when assessing the renal function in order to decide correct dosage of renal eliminated drugs [19]. Decline in kidney function with age is probably the reason why NHR in our study, in line with others [1, 28], had a wider range than the values of NORIP raw origin for sodium. However, this change does not appear to be of clinical relevance. Like others [1, 28] we found decreased levels of albumin for the NHR. This is probably due to a decline in organ functions and inflammatory processes [35]. Thus, low values of albumin may not to be reliable as an indicator of malnutrition in frail elderly. We found increased urea in NHR, as others [1, 28], which may be a natural part of the ageing process. However, as a consequence conditions with increased diuresis due to high water and/or low supply of amino acids could be missed.

The number of NHR without disease was only nine. They did not show any difference in levels of the investigated analytes, indicating that changed levels of biomarkers are due to an ageing, and not the presence of diseases. Changes related to ageing, as well as diseases that can arise with increased age, certain analytes are changed and some of them changes more than others. The assessment is often complex because different diseases occurs in different number and in different combinations. The present results show that current reference intervals can be misleading when assessing frail elderly individuals. The limited number of included NHR, is however not sufficient to create new reference intervals for this group.

Limitations of the present study are that plasma from the reference populations and the frail NHR have been analysed at different accredited laboratories using measurement methods from different manufactures. Nevertheless, all of them participated in the Swedish national system for external quality control (EQUALIS) showing low bias. A majority of the NHR suffered from heart disease. Unfortunately the present study was restricted to the use of EDTA plasma and therefore excluded analysis of biomarkers of interest in cardiac disorders.

In summary, we found that NHR differ in values of IgG, IgM, C3, C4, ALT, albumin, creatinine, Na, phosphate and urea compared with values from current reference populations. Whether values outside current reference intervals in elderly persons reflect disease processes, or are related to consequences of ageing, like general decline in organ functions, are unclear and need to be further studied.

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Figure 1: *Distribution of Immunoglobulin (Ig)G ($p<0.05$), IgM ($p<0.001$), Complement factor (C)3 ($p<0.001$) and C4 ($p<0.001$) levels for nursing home residents (NHR) and blood donors. P-values within brackets above denote differences between NHR and blood donors (Student´s t-test). Median and variation between the 25: th and 75: th percentiles are presented in the box and whiskers indicate minimum and maximum. Circles represent values between 1.5 and 3 times the interquartile range and * represent values that are more than 3 times the interquartile range.*

Figure 2: *Distribution of alanine aminotransferase (ALT) ($p<0.001$), albumin ($p<0.05$), creatinine ($p<0.001$), phosphate ($p<0.001$, NHR and NORIP raw origin), sodium ($p<0.01$) and Urea ($p<0.001$) levels for nursing home residents (NHR) and Nordic Reference Interval Project (NORIP) raw origin 80 and raw origin. P-values within brackets above denote differences between NHR and NORIP raw origin 80 as well as NORIP raw origin (Student´s t-test). Median and variation between the 25: th and 75: th percentiles are presented in the box and whiskers indicate minimum and maximum. Circles represent values between 1.5 and 3 times the interquartile range and * represent values that are more than 3 times the interquartile range.*

Table 1: Distribution of chronic diseases, ordered analgesic drugs and nutritional status, dependency in daily living, divided into gender, in 138 nursing home residents ≥ 80 years. More than one disease could occur for each participant.

Condition	Women (%) n = 91	Men (%) n = 47
Chronic heart disease	64.1	76.6
Dementia	32.6	19.1
Stroke	21.7	27.7
Diabetes mellitus type 2	19.6	12.8
Kidney disease	1.1	0
Liver disease	0	2.1
Autoimmune disease	4.3	6.4
ADL[a] > 5	60.9	38.3*
Malnutrition	16.5	12.8
Paracetamol > 3 g/day	45.1	40.4
COPD[b]	1.1	8.5*

*p<0.05; [a]Activities of daily living [32];[b]Chronic obstructive pulmonary disease

Table 2: Reference intervals for immune parameters

Component	Unit	Age	Reference intervals
IgA	g/L	> 6 years	0.7 – 3.7
IgG	g/L	> 6 years	6.0 – 15.0
IgM	g/L	> 6 years	0.4 – 2.2
C3	g/L		0.7 – 1.3
C4	g/L		0.13 – 0.32

Table 3: Reference values proposed by NORIP (Nordic Reference Interval Project)

Component	Unit	Sex	Reference intervals
ALT	µkat/L	F	< 0.76
		M	< 1.2
Albumin	g/L	F, M	34 – 45
AST	µkat/L	F	< 0.61
		M	< 0.76
γ-GT	µkat/L	F	< 1.3
		M	< 2.0
LD	µkat/L	F, M	< 4.3
Phosphate	mmol/L	F	0.8 - 1.5
		M	0.75 - 1.4
Sodium	mmol/L	F, M	137 – 145
Urea	mmol/L	F	3.1 - 7.9
		M	3.5 - 8.2
Creatinine	µmol/L	F	45 – 90
		M	60 - 105

F: female M: male

Table 4: Differences in mean levels in individuals with or without mentioned condition.

Analyte	Anti-depressants		Paracetamol ≥3 /day		Malnutrition		ADL[a] index		Dementia		Heart disease		Autoimmune disease	
	Yes n=38	No n=99	Yes n=60	No n=78	Yes n=21	No n=115	< 5 n=58	≥ 5 n=74	Yes n=38	No n=99	Yes n=69	No n=37	Yes n=5	No n=101
IgG (g/L)			10.6	12.0*										
C3 (g/L)			1.52	1.34**			1.36	1.47*						
C4 (g/L)			0.37	0.33*			0.31	0.38**						
			n=44	n=62			n=58	n=73						
ALT (µkat/L)			0.17	0.14**										
Albumin (g/L)	36.3	37.6*			34.5	37.7**	38.7	36.1	35.6	37.9**				
Creatinine (µmol/L)											115.2	93.9**		
Urea (mmol/L)											9.7	7.4**	7.2	9.0*

*p<0.05, **p<0.01. [a]Activities of daily living,