Circulating levels and assessment of
clinical laboratory analytes, in ≥ 80 -year-old,
apparently healthy, moderately healthy,
and frail individuals

Maria Edvardsson

Linköping University Medical Dissertation

No. 1682

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Linköping 2019

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Printed in Sweden by LiU-Tryck, Linköping, Sweden, 2019

ISBN: 978-91-7685-076-3

ISSN: 0345-0082

"Ju mer jag lärde mig, bestå mer insåg jag hur lite jag egentligen begrep"

Pappa

Abstract

Blood samples are often used to investigate the possible presence of disease and to make treatment decisions. In the interpretation of the results, comparison either with previous values from the same individual or with a set of appropriate group-based reference intervals are used. Current reference intervals for common laboratory analytes are often based on measurements from apparently healthy persons aged 18–65 years. Age is accompanied by a general decline in organ functions and it is difficult to determine whether a change in levels of laboratory analytes in an elderly individual can be attributed to age alone, independent of environmental or disease processes. Frailty can be seen as a consequence of age-related multifactorial deterioration – physical, cognitive and sensory – resulting in vulnerability and lack of adaptability to internal stressors such as infection or new medication and/or external stressors such as fall at home. Consensus about the definition of “frail” and “frailty” is missing, both nationally and internationally, the question arises whether different definitions of “frailty” affect the interpretation of analytes when comparing different groups of elderly.

The overarching aim of the thesis was to interpret and assess circulating levels of some clinical laboratory analytes in relation to conventional reference values in ≥ 80 -year-old, “apparently healthy”, “moderately healthy”, and “frail” individuals.

Data originated from other studies, in which blood samples were collected from individuals ≥ 80 -year-old. Comparisons in Paper I of levels of some laboratory analytes, from 138 nursing home residents (NHRs), was made with blood from reference populations, both blood donor and the NORIP study. The results indicated differences for some immunological (complement factor 3 and 4, immunoglobulin G and M) and chemical analytes (alanine aminotransferase (ALT), phosphate, albumin, sodium, creatinine and urea), but no differences in levels occurred for aspartate aminotransferase (AST), gamma-glutamyltransferase (γ -GT) or lactate dehydrogenase (LDH). It was unclear whether the differences were due to differences in age between the elderly and the reference populations or whether the elderly individuals had chronic diseases and were on medication. In Paper II, 569 individuals elderly individuals ≥ 80 years old were classified as “healthy”, “moderately healthy”, and “frail”, based on diseases, medications and physical and cognitive abilities. Statistical differences between the groups were found for the investigated analytes; albumin, ALT, AST, creatinine and γ -GT. In Paper IV, individuals from Paper II ($n=569$) were divided into two groups and thereafter divided into “apparently healthy”, “moderately healthy”, and “frail”. One group was subdivided into

“apparently healthy”, “moderately healthy” and “frail” based on physical and cognitive abilities and the other group was divided based on the frailty index (FI). There was no statistical difference found between “apparently healthy” and “moderately healthy” groups, regardless of classification model used. Among “frail” individuals, differences in levels occurred for three out of the five investigated analytes: ALT, creatinine and γ -GT, with lower levels occurring when the FI classification model was used. No differences in levels occurred for albumin or AST in “frail” individuals, regardless of classification model used. The aim of Paper III was to study whether 1-year changes in complete blood count (CBC) (including haemoglobin (Hb), red blood cell (RBC), erythrocyte volume fraction (EVF), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), white blood cell (WBC) and platelet count (PLT)), C-reactive protein (CRP) and interleukin (IL)-1 β , IL-1RA, IL-6, IL-8 and IL-10 are associated with survival in elderly NHRs aged ≥ 80 years. Elevated levels of CRP and IL-8 during 1-year follow-up were associated with reduced length of survival in elderly NHRs. Based on the present thesis it is clear that there is need for reference intervals that consider both age and health status in elderly individuals. A reasonable conclusion when interpreting levels of analytes in elderly individuals with disease or frailty is that individual evaluation based on the individual’s previous levels, is recommended.

Sammanfattning

Blodprover används ofta för att undersöka ev förekomst av sjukdomar och för att fatta behandlingsbeslut. Vid tolkningen av resultaten används jämförelse antingen med tidigare värden från samma individ eller med en uppsättning lämpliga gruppbaseade referensintervall. Nuvarande referensintervall för vanliga laboratorieanalyser baseras ofta på mätningar från tillsynes friska personer i åldern 18–65 år. Åldern åtföljs av en allmän nedgång i organfunktioner och det är svårt att avgöra om en ev förändring av nivåerna av laboratorieanalyterna kan enbart beror på skillnaden i ålder, oberoende av miljö- eller sjukdomsprocesser. Skörhet kan ses som en konsekvens av åldersrelaterad multifaktoriell försämring - fysisk, kognitiv och sensorisk - vilket resulterar i sårbarhet och brist på anpassningsförmåga till interna stressfaktorer som infektion eller ny medicinering och/eller yttre stressorer, såsom att ramla hemma. Konsensus om definitionen av "skörhet" saknas, både nationellt och internationellt och frågan uppstod om olika definitioner av "skörhet" påverkar tolkningar och referensintervall för laboratorieanalyser, när man jämför olika grupper av äldre individer.

Det övergripande syftet med avhandlingen var att tolka och bedöma cirkulerande nivåer för några kliniska laboratorieanalyser i förhållande till gällande referensvärden hos ≥ 80 -åriga, "hälsosamma", "måttligt friska" och "sköra" individer.

Data kommer från andra studier, inom vilka blodprov samlades, alla från individer ≥ 80 år. Jämförelser i studie I gjordes mellan blodprover från 138 individer i särskilt boende, med blodprover från referenspopulationer, både blodgivare och från NORIP-studien. Resultaten visade skillnader för vissa immunologiska (komplementfaktor 3 och 4) och kemiska analyser (alaninaminotransferas (Alat), fosfat, albumin, natrium, kreatinin och urea), men inte alla (aspartataminotransferas (Asat), gamma-glytamytransferas (γ -GT) eller laktatdehydrogenas (LD)). Det var oklart om skillnaderna berodde på skillnader i ålder mellan de äldre och referenspopulationerna eller om de äldre individerna hade kroniska sjukdomar och medicinerade. I studie II klassificerades 569 individer ≥ 80 år som "hälsosamma", "måttligt friska" och "sköra", baserat på sjukdomar, medicinering och fysiska och kognitiva förmågor. Statistiska skillnader mellan grupperna hittades för de undersökta analyterna: albumin, Alat, Asat, kreatinin och γ -GT. I studie IV delades individer från papper II ($n = 569$) in i två grupper och delades därefter upp i "hälsosamma", "måttligt friska" och "sköra". En grupp delades in i "hälsosamma", "måttligt friska" och "sköra" baserat på fysiska och kognitiva förmågor och den andra gruppen delades in baserat på skörhetsindex. Det fanns ingen

statistisk skillnad mellan ”hälsosamma” och ”måttligt friska” grupperna, oavsett vilken klassificeringsmodell som användes. Bland ”sköra” individer inträffade skillnader i nivåer för tre av de fem undersökta analyterna: Alat, kreatinin och γ -GT, med lägre nivåer där skörhetsindex hade använts som klassificeringsmodell jämfört klassificering baserad på fysiska och kognitiva förmågor. Syftet med studie III var att studera om 1-års förändringar i blodstatusparametrar (hemoglobin (Hb), erytrocytpartikelkoncentration (EPK), erytrocytvolymfraction (EVF), medelcellvolum (MCV), mean corpuscular Hb concentration (MCHC), leukocytpartikelkoncentration (LPK) och trombocytpartikelkoncentration (TPK)), C-reaktivt protein (CRP) och interleukin (IL)-1 β , IL-1Ra, IL-6, IL-8 och IL-10 var associerade med överlevnad hos individer från särskilt boende ≥ 80 år. De mest framträdande resultaten var att förhöjda nivåer av CRP och IL-8 under 1-års uppföljning var förknippade med förkortad överlevnadstid hos äldre från särskilt boende.

Baserat på den aktuella avhandlingen är det tydligt att det finns behov av referensintervall som beaktar både ålder och hälsostatus hos äldre individer. En rimlig slutsats när man tolkar nivåer av laboratorieanalyser hos äldre individer med sjukdom eller skörhet är att individuell utvärdering baserad på individens tidigare nivåer rekommenderas.

List of publications

This thesis is based on the following papers referred to in the text by their Roman numerals.

- I. Maria Edvardsson, Märtha Sund-Levander, Jan Ernerudh, Elvar Theodorsson, Ewa Grodzinsky. Clinical use of conventional reference intervals in the frail elderly. *J Eval Clin Pract.* 2015;21(2):229-35.
- II. Maria Edvardsson, Märtha Sund-Levander, Anna Milberg, Ewa Wressle, Jan Marcusson, Ewa Grodzinsky. Differences in levels of albumin, ALT, AST, γ -GT and creatinine in frail, moderately healthy and healthy elderly individuals. *Clin Chem Lab Med.* 2018;56(3):471-8.
- III. Maria Edvardsson, Märtha Sund-Levander, Anna Milberg, Jan Ernerudh, Ewa Grodzinsky. Elevated levels of CRP and IL-8 are related to reduce survival time: 1-year follow-up measurements of different analytes in frail elderly nursing home residents. *Scand J Clin Lab Invest.* 2019;10:1-5.
- IV. Maria Edvardsson, Märtha Sund-Levander, Anna Milberg, Jan Ernerudh, Ewa Wressle, Jan Marcusson, Ewa Grodzinsky. Classification of ≥ 80 -year-old elderly individuals into “healthy”, “moderately healthy” and “frail” based on different frailty scores, affects the interpretation of laboratory results. *Manuscript*

Abbreviation list

ADL	Activities of daily living
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APC	Antigen presenting cell
AST	Aspartate aminotransferase
BCP	Bromocresol purple
C	Complement factor
CBC	Complete blood count
CHMS	Canadian health measure survey
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
DC	Dendritic cell
DEKS	Danish institute for external quality assurance for laboratories in health care
DNA	Deoxyribonucleic acid
EA	European co-operation for accreditation
EDIS	Early detection of infection scale
EDTA	Ethylene diamine tetra-acetic acid
ELSA	Elderly in Linköping screening assessment
EVF	Erythrocyte volume fraction
FCA	Federal council of aging
FI	Frailty index
γ -GT	gamma-glutamyltransferase
Hb	Haemoglobin
IADL	Instrumental ADL
IAM	Instrumental activities measure
IFCC	International federation of clinical chemistry
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor-1
IL	Interleukin
IL-1RA	Interleukin receptor antagonist
ILAC	International laboratory accreditation cooperation
ISO	International organisation for standardization

IVD	In vitro diagnostic
LDH	Lactate dehydrogenase
MCHC	Mean corpuscular Hb concentration
MCV	Mean corpuscular volume
MMSE	Mini-mental state examination
NK	Natural killer
NORIP	Nordic reference interval project
PADL	Personal ADL
PLT	Platelet count
RBC	Red blood cell
ROS	Reactive oxygen species
SD	Standard deviation
SWEDAC	Sweden's national accreditation body
Tc	Cytotoxic T cell
TGF- β	Transforming growth factor beta
Th	T-helper cell
T reg	Regulatory T cell
WBC	White blood cell
WHO	World health organization

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Introduction

Blood samples are often used to investigate the possible presence of disease and to make treatment decisions. In the interpretation of the results, comparison either with previous values from the same individual or with a set of appropriate group-based reference intervals are used. Current reference intervals for common laboratory analytes are often based on measurements from apparently healthy persons aged 18–65 years. Age is accompanied by a general decline in organ functions, in particular cardiovascular, pulmonary and kidney functions. It is difficult to determine whether the change is ascribable to age alone, independent of environmental or disease processes. 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, malnutrition and the use of medication. Frailty can be seen as a consequence of age-related multifactorial deterioration – physical, cognitive and sensory – resulting in vulnerability and lack of adaptability to internal stressors such as infection or new medication and/or external stressors such as fall at home. Consensus about the definition of “frail” and “frailty” is missing, both nationally and internationally, the question arises whether different definitions of “frailty” affect the interpretation and reference intervals of analytes when comparing different groups of elderly. When developing reference intervals and studying elderly individuals, there is a high risk that moderately healthy or frail elderly people are excluded while an elite of the healthiest individuals remains, according to current routines.

A report from 2017, by the Director-General of the World Health Organization (WHO), about the evolution of global public health over the last decade states that “health and life expectancy have improved nearly everywhere” (1). Increased life expectancy results in more healthy years, which is a big benefit for the person in question, and their relatives and friends, but also for society as the person can be in work and active for more years than in the past (2).

With ageing, some well-adjusted regulatory systems in the immune system do not act in their well-adjusted way any longer and the result is chronic low-grade production of proinflammatory molecules, with tissue injury as a consequence. Elderly people also have a greater risk than younger individuals of being affected by infections whose signs and symptoms are often non-specific (3, 4). Early detection of infectious disease improves the possibility of early treatment in the person’s home environment, which increases the

possibility of maintaining physical function and therefore wellbeing. Early diagnosis and treatment of infectious disease is important for the individual as it reduces personal suffering; but it is important also from a health economic perspective.

Laboratory analytes are amongst the diagnostic cornerstone on which caregivers rest when they confirm or deny the presence of diseases. However, there is a lack of knowledge regarding the effects of natural ageing in general and how it affects the levels of laboratory analytes in particular. The present thesis emphasizes that this approach may cause problems when laboratory results from elderly individuals with chronic disease and on chronic medication use are being interpreted. The insufficient knowledge regarding natural ageing and/or how it affects the levels of analytes is a strong argument for studying at least the most common analytes in relation to health and disease in ≥ 80 -year-old individuals.

Background

Reference intervals

History

The development of the methods for establishing and interpreting reference intervals began during the years 1965–1980, when laboratories thought technical developments became able to produce large amounts of high quality data (5). During that period biological and analytical variations were studied, together with intra- and inter individual variability and pre-analytical factors, like which anticoagulants to use, affecting the values (6-8). In 1969 a new concept of reference intervals was launched as the former concept of normal values/range was considered flawed (9). Normal values are calculated based on Gaussian distribution, which is not necessarily representative of the distribution of all analytes. The word “normal” also has many meanings: in the medical context, it is closely associated with health, i.e. being healthy, or non-pathological. Other meanings include “common”, “frequent”, “occurring as a rule”, “not deviating or disturbing”, “conforming to the norm or regulations” (e.g. in NTP = normal temperature and pressure), and so on (9). A well-defined nomenclature for “reference values”, “reference intervals” and “recommended procedures” was clearly needed. In response, the Nordic Society for Clinical Chemistry founded its Committee on Reference Values. Soon after, the

International Federation of Clinical Chemistry (IFCC) formed an Expert Panel on Reference Values (9) also with a strong Nordic representation. The concept of reference intervals is essentially philosophical and can be expressed as the establishing and using of relevant data for interpreting medical observations.

The idea to establish rules to produce, issue and use reference data for decision making in clinical and preventive medicine was easy; achieving this goal in practice was more challenging (9). “Reference” could mean values from healthy persons or values from the general, non-hospitalized population; they could be from persons in their optimum state of health (such as at between 20 and 30 years of age) or they could be the conveniently obtained hospital population values from which the most obvious outliers are eliminated. The principal aims of medicine are to make or keep people well, to help them to achieving health and to support them in retaining it (9). These aims require that the analyte levels of healthy individuals are known. Production of reference intervals of this kind requires large, healthy populations, which often involve military recruits, blood donors, medical students, and laboratory workers etc., i.e. populations that are sometimes poor representatives of the healthy populations (5, 10).

During the 80s and 90s, there was a period of development but hardly any efforts were made to develop the practical use of reference intervals by clinicians (5). One investigation during this period recruited 1,000 families to study individual specific reference values in subjects under medication, e.g. contraceptive pills (11). Also attempts were made to propose new reference limits for a better use in preventive health maintenance including for and by the patients themselves. Data produced were not limited to clinical chemistry measurements but also included weight, blood pressure, etc. (11). Various scientific societies contributed during that period of 20 years and they had the merit to disseminate the concept of reference values. Thanks to these scientific societies, reference intervals have become a basic tool for the interpretation of the quantitative results of laboratory analytes (5). However, the evolution of medical practice led to questioning of the current relevance of the concept of reference intervals (12). By the late 1990s, the concept of reference intervals were progressively used by all health professionals, including clinical chemists and clinicians, and simultaneously by all official bodies in charge of legislation (5). Its use by the in vitro diagnostic (IVD) industry and laboratorians is now recommended by the European Directive 98/79 EC (12) and the International Organization for

Standardization (ISO) 15189 standard (13). In contrast, the application of the concept in clinical and laboratory practice remained difficult, and procedures for estimating reference intervals needed to be improved (5). The recommendations were too long and too expensive and not feasible for all laboratories. In the year 2000, a group of scientists and professionals focused on areas for improving the use of reference values, by calling for the development of practical recommendation guidelines e.g. when selecting proper reference populations and on the statistical methods to be used (14).

Today, common criteria when establishing reference intervals for blood samples are to ask whether the person is healthy (9) and to exclude individuals with disease and/or on medication (15-17). The complexity of the problem, however, became apparent when the Scandinavian Committee on Reference Values took the WHO's 1948 definition of "health" literally, and individuals with diagnoses were deemed not to be healthy and were therefore excluded from the reference population (18). The WHO definition starts as follows: "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." (19). According to the "biostatistical theory of health", a disease is a "type of internal state which is either an impairment of normal functional ability, i.e. a reduction of one or more functional abilities below typical efficiency, or a limitation on functional ability caused by environmental agents" and health is identical with the absence of disease (20). The "holistic theories" refer not only to survival, but also to quality of life (20). Testing of this recommendation showed that such "healthy" persons are a minority in the population, and that in practice this approach to diagnose "health" is near impossible (21). To indicate that levels are derived from persons considered to be healthy, the term "health-related (or health-associated) reference intervals" has been proposed (9). Also, the IFCC recommendation, Part 1, states that the term "reference" (as in "reference value", "reference interval", etc) should be preceded by a word qualifying the state of health (22). Care should be taken to use appropriate nomenclature. Terms such as "goal values" or "optimum values" may be considered (9).

Calculation of reference intervals

The selection of a reference population for developing reference intervals has been approached from many different angles, based on different philosophies, needs and available resources (15). "Apparently healthy" people, blood donors aged 18–65 years or hospital staff are still the most commonly used reference population (10).

To assess whether levels of an analyte have changed spontaneously or as a result of therapy, the level can be done by comparing current levels with previously observed levels from the same individual, or with a set of appropriate group-based reference intervals (9, 23). The group-based reference values are often condensed into a reference interval defined by two reference limits (24).

For establishing group-based reference intervals, the procedures may differ, from simple intuitive estimation of the available data to complex statistical techniques. A common convention is that the reference interval should be within the central range of the reference distribution (24), usually between the 2.5th and the 97.5th percentiles. Use of alternative fractions or an asymmetric location of the reference interval may in some cases be more appropriate, however (24). Using standard methods based on Gaussian theory, by calculating the mean ± 2 standard deviation (SD), if the values are normally distributed, is an alternative method. However, with biochemical markers, the values are not always normally distributed, and in some cases the square root or log transformation is used to produce a reference interval (10, 25).

Some key factors to take into consideration when selecting a reference sample are that the individuals included in the reference sample should be as similar as possible to the individuals whose analyte concentrations are to be determined, with the exception of the present disease (26). The IFCC and the Scandinavian Committee on Reference Values have adopted strict rules for generating reference intervals, which have been presented in six parts. The first part contains definitions of terms used in the production of reference values, such as “reference individual”, “reference population”, “reference value”, and so on (22). How to make a proper selection of individuals for the production of reference intervals is described in the second part (15). The third part describes the preparation of individuals and the procedure for collection of specimens (27). Control of analytical variation in the production, transfer and application of reference intervals is described in the fourth part (28). Finally, the statistical treatment of collected reference values and the presentation of observed values in relation to reference intervals, respectively, are described in the fifth and sixth parts (24, 29).

RefVal (Department of Clinical Chemistry, Rikshospitalet, N-0027 Oslo, Norway), is a computer program that implements the recommendations of the IFCC on the statistical

calculation of reference values (30). It presents the lower reference limit as the 0.025 fractile and the upper limit as the 0.975 fractile, as well as the 0.90 confidence interval of fractile. The estimation of confidence intervals is only possible when the sample size is adequate. For example, in RefVal, a 90% confidence interval requires a sample size of at least 119. RefVal checks for and handles outliers and skewness and if the data fit Gaussian distribution, RefVal implements both the non-parametric and the parametric methods for estimation of reference limits recommended by the IFCC. As empirical distributions in laboratory medicine rarely are Gaussian, mathematical transformation of these data is needed. First skewness should be removed, and then data should be adjusted for remaining non-Gaussian kurtosis. These goals may be attained by several mathematical functions, and the functions recommended by the IFCC and use of RefVal is considered to have great flexibility and reliability (30).

Standardization and accreditation

The calibration of methods performed by laboratory analyzers is performed “by comparison” with a standard source of known levels (31). To determine the performance of an instrument, a number of calibration reference sources can be used to cover the working range of the instrument under calibration. However, the question should always be asked, what is it being measured against? In other words, what is the measurement reference or “gold standard”? Internal controls are used within laboratories to investigate whether a method is stable or not, but external controls are also used. The purpose with external controls is to ensure that the methods correspond between laboratories and also between countries (32). These controls are also often used to follow up on the implementation of standardizations.

Accreditation is an independent, objective method of reviewing calibration facilities, procedures and staff. A third party, usually a government authority, e.g. in Sweden, Sweden’s national accreditation body (Swedac), is tasked with making products and services safe and reliable (33). Swedac in turn is a member of the European co-operation for Accreditation (EA) (34), which is an organization for accreditation of laboratories and is in turn accredited by the International Laboratory Accreditation Cooperation (ILAC) (35). The ILAC is an international organization for accreditation bodies operating in accordance with ISO/IEC 17011 and is involved in the accreditation of conformity assessment bodies including e.g. calibration laboratories as per ISO/IEC 17025 (35).

Swedac regularly reviews and assesses the certificated staff, records, standards, procedures such as how often controls are performed, coefficients of variation (CVs) from the control results both within and between laboratories, and also procedures used to carry out the calibration. Third-party assessment ensures that the calibration provider produces a traceable and therefore internationally accepted calibration service. Accreditation meeting the international standard ISO-17025 (36) means that the calibration provided to the user is demonstrably traceable, to, e.g., CRM470 for plasma proteins and SRM967 for creatinine, by an unbroken chain of measurements. Besides Sweden, the other Nordic countries have their own organizations that ensure the quality of the laboratory analytes provided within the countries (37-39). This means that the analytes can be compared with each other, even if they are analysed at different laboratories with different laboratory devices.

Reference intervals and elderly

The elderly population is usually excluded from reference samples, as they often suffer from disease and use medications. Some attempts have been made to establish reference intervals for laboratory analytes in elderly individuals and different approaches have been used (Table 1). To this end, some groups use rigorous health screening to investigate disease-free individuals (40-42), while others have used individuals already participating in other studies (10, 43-45). Furthermore, some have collected blood samples from individuals visiting physicians' offices, primary health care centers or outpatient clinics during a specific time period (46, 47). None of these attempts (shown in more detail below) has to our knowledge yet resulted in the routine use of specific reference intervals for the elderly.

Publications found in PubMed in May 2019, using the key words "reference interval" AND "elderly", are presented in Table 1. Limitations were set for "humans", publications in "English" and "aged: 65+ years". Also, other publications that were referred to from the once found in PubMed, were included.

Table 1: Previous studies of laboratory analytes in different age groups and reference intervals proposed for different age groups. Key words used in PubMed were “reference interval” AND “elderly”. Limitations were set including “humans”, publications in “English” and “aged: 65+ years”. Also publications related to them found in PubMed, were included.

	N	Age	Inclusion criteria	Exclusion criteria	Results
Bohnen N, Degenaar CP, Jolles J. Influence of age and sex on 19 blood variables in healthy subjects. <i>Z Gerontol</i> 1992;25 (5), 339–345. (40)	80	Four age groups; 20–80 years	Disease-free persons who had undergone rigorous health screening.	Unclear description	Of 19 analytes, four differed in levels between age groups and six showed both age and sex differences.
Back SE, Nilsson JE, Fex G, et al. Towards common reference intervals in clinical chemistry. An attempt at harmonization between three hospital laboratories in Skane, Sweden. <i>Clin Chem Lab Med</i> 1999;37 (5), 573–592. (10)	255	20.1–88.5 years	Randomly selected population from Kristianstad, Sweden.	Separate exclusion criteria were defined for each analyte. Data on pregnant women and non-Caucasian subjects were excluded.	Reference intervals for 70 analytes were calculated, some for men and women separately and for different age groups.
Kallner A, Gustavsson E, Hendig E. Can age and sex related reference intervals be derived for non-healthy and non-diseased individuals from results of measurements in primary health care? <i>Clin Chem Lab Med</i> 2000;38 (7), 633–654. (46)	Population of the catchment area of Karolinska Hospital, Stockholm, Sweden; at the time about 350,000 individuals.	0–98 years	Levels of analytes from patients visiting primary health care, physicians’ offices and outpatient clinics at Karolinska Hospital during 18 consecutive months were studied retrospectively.	Incorrect, incomplete or confidential identification, e.g. from patients with certain diagnoses (mainly psychiatric and venereal)	Results from patients included once in the database were selected, called “non-diseased”. Medians and 98th percentiles were calculated, levels outside the 98th percentile were excluded before the final medians and 95th percentiles were calculated and formed reference intervals for 37 analytes.
Lock RJ, Unsworth DJ. Immunoglobulins and immunoglobulin subclasses in the elderly. <i>Ann Clin Biochem.</i> 2003;40 (2), 143–148. (47)	2,071	Three age groups: 18–60, 61–70 and 71+ years	Consecutive serum samples from patients who had been referred routinely for immunoglobulin (Ig) analysis during 2000, Southmead Hospital, Bristol, UK	First-received specimens for each patient were used, duplicates excluded.	Reference ranges, separated by age group, for IgA, IgG, IgM, IgG1, IgG2, IgG3 and IgG4 were defined non-parametrically as the 2.5th to 97.5th centiles.
Nilsson SE, Ervin PE, Tryding N, et al. Biochemical values in persons older than 82 years of age: report from a population-based study of twins. <i>Scand J Clin Lab Invest</i> 2003;63 (1), 1–13. (43)	535	82–99 years	Data from the longitudinal Swedish study titled “Origins of Variance in the Old-Old: Octogenarian Twins (OCTO-Twin)”. All same-sex twin pairs aged 80 years and older were identified as potential participants.	Unclear description	Survival over a 6-year period was used as a reference for overall health. Increased mortality was indicated for subjects of both genders with high serum levels of urea, urate, gamma glutamyltransferase (γ -GT), free thyroxin and plasma homocysteine.

Rustad P, Felding P, Franzson L, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. <i>Scand J Clin Lab Invest</i> 2004;64:271–284. (41)	3,036	18–90 years	Being subjectively healthy; not being pregnant or breastfeeding; not having been seriously ill during the past month; not having consumed more than 24 g pure alcohol in the last 24 hours; not having given blood as a donor in the past 5 months; not having taken prescribed drugs other than oral contraceptives or oestrogens during the past 2 weeks; not having smoked during the hour before blood sampling.	-Glucose ≥ 11.1 mmol/L, fasting glucose ≥ 7.0 mmol/L (fasting ≥ 12 h). -5s/3s and 4s/4s rule: At least one value outside median; 5s for one property and at least one value for a different property outside median; 3s (5s 3s rule). The same rule has also been applied with 4s limits for both properties (s is the total biological variation based on NORIP data, logarithmic transformations).	Reference intervals for 25 common biochemical properties were provided. Calculations were done using the computer program RefVal 4.0.
Huber KR, Mostafaei N, Stangl G, et al. Clinical chemistry reference values for 75-year-old apparently healthy persons. <i>Clin Chem Lab Med</i> 2006; 44 (11), 1355–1360. (42)	606	75-year-olds	Negative history and clinical chemistry analysis for diseases concerning: heart, thyroid, liver, kidney, diabetes, neurological or other relevant diseases, such as cancer.	Unclear description	Reference values for 75-year-old apparently healthy individuals were given as 2.5 th and 97.5 th percentiles with 90% confidence intervals for 45 clinical chemistry analytes.
Adeli K, Higgins V, Nieuwesteeg M, et al. Biochemical marker reference values across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian health measures survey. <i>Clin Chem</i> 2015;61:1049–1062. (44)	11,999	3–79 years	Participants in the Canadian Health Measure Survey (CHMS), representing approximately 96% of Canada's population.	Pregnancy, diagnosed serious medical illness or chronic conditions, or use of prescription medication	Age- and sex-specific reference intervals for 24 chemistry analytes were calculated.
Helmerson-Karlqvist J, Ridefelt P, Lind L, et al. Reference values for 34 frequently used laboratory tests in 80-year-old men and women. <i>Maturitas</i> 2016;92:97–101. (45)	1,016	80-year-olds	Seventy-year-old individuals living in Uppsala, Sweden, were originally included in the Prospective Investigation of the Vasculture in Uppsala Seniors (PIVUS) study. This study reports the reinvestigation of the cohort at the age of 80 years.	Persons with a known diagnosis of diabetes or fasting glucose value ≥ 7.0 mmol/L were excluded.	Reference intervals for 34 laboratory analytes are provided. Calculations of reference intervals were performed using the computer program RefVal 4.0.

The immune system

Organisms that are multicellular are exposed to outside invaders such as viruses or to changed components in the host, such as cancerous cells (12). The organism needs a reliable immune system to take the fights that are needed. The innate immune system is the first line of defense, and the adaptive system is a more specific response to repeated challenges resulting in immunological memory.

The innate immune system

Monocytes settle in the tissue, mature and become macrophages and phagocytose (cell-eating) foreign particles as microbes and macromolecules, as well as body tissues that are injured or dead (Figure 1) (31). Macrophages also act as antigen presenting cells (APCs) and therefore also play an important role in immunity. Natural killer (NK) cells are large lymphocytes found in blood, lymphoid tissues and spleen. They contain numerous cytoplasmatic granules that are capable of lysing tumour and virus-infected cells. Granulated leukocytes, i.e. neutrophils, eosinophils and basophils, are cells containing abundant cytoplasmic granules. Neutrophils are the most numerous leukocyte type in blood and respond rapidly to phagocytosis or chemotactic stimuli (recruit phagocytes to the infection site) and can be activated by cytokines, see below. Dendritic cells (DCs) are thought to be the main APC that migrate to infection sites in most tissues in the body. They have a central role in driving immune responses since they are efficient at presenting antigens to T cells in lymphoid organs (48).

The adaptive immune system

T cells are lymphocytes that arise in the bone marrow and then migrate to the thymus, where they mature into the CD^4 + T-helper (Th) cells and CD^8 + cytotoxic (Tc) cells (31). There are four main subsets of Th cells: Th₁, Th₂, Th₁₇, and regulatory T cells (T regs) (Figure 1). The activities of the adaptive immune system are regulated by signals from Th cells. Their signals also regulate the activities of the cells in the innate immune system. Th cells induce most of their helper functions by secreting cytokines, see below. Cytokines produced by Th₁ cells activate macrophages and the cytotoxic lymphocytes, resulting in a cell-mediated immune response against viruses and intracellular bacteria. Cytokines produced by Th₂ cells help to activate mast cells and eosinophils, resulting in

anti-parasitic responses. T regs are cells actively suppressing activation of the immune system and preventing pathologic self-reactivity by producing interleukin (IL)-10 and transforming growth factor beta (TGF- β). B cells migrate from the bone marrow to peripheral organs where they mature. B cells with the same specificity on exposure and later often develop into plasma cells, which actively secrete antibodies. The complement system constitutes a part of the innate as well as the adaptive immune system. The complement system can e.g. be activated at contact with microorganisms and enhance phagocytose and induce cytotoxicity (48).

See: List of abbreviations

Figure 1: Cells and substances in the innate and adaptive immune system. Figure reproduced with permission from Jonny Hallberg, Sjöbo, Sweden.

Immunoglobulins

The protective effects of humoral immunity are mediated by a family of structurally related glycoproteins called antibodies, or immunoglobulins (Igs) (49). There are five different classes of Igs, placed at different places within the body: IgAs placed on the mucous membranes; IgDs and IgGs in the bloodstream; IgEs at mastcells and basophiles and IgMs in the tissue. Both Th₁ and Th₂ cells initiate the humoral immune system by activating naïve B cells to produce antibodies and induce Ig's class switching (49).

Interleukins

One major way in which cells of the immune system communicate with one another and with other cells are by the use of soluble cytokines. Some are referred to as interleukins, that the molecules that act between (inter) leucocytes, a name that is incorrect as many interleukins both are formed and affects cells, outside the immune system. There are different kinds of cytokines like pro- inflammatory, cytokines that regulate lymphocyte activation, growth and differentiation, anti-inflammatory cytokines, and cytokines with both pro- and anti-inflammatory activity (49).

Biological theories of ageing

In the biological theories of ageing, what causes ageing is often considered to be stochastic or genetic. Stochastic theory of ageing suggests that small random failures or changes that occur and accumulate over time can damage cells and tissues, which can lead to a decline in the function of an organ (50). The genetic control of ageing is multifactorial. Studies of twins estimate that genetic factors contribute to 20–30% of ageing. According to programmed theories, there is a biological clock that governs development, growth, maturity and ageing by switching genes on and off. It appears that ageing genes slow and stop biochemical pathways (50).

Stochastic theories of ageing

The stochastic theories of ageing include the wear and tear theory, which proposes that cells and tissues have vital parts that wear out over time, resulting in ageing. Like components of an ageing car, parts of the body eventually wear out from repeated use and die, and eventually the whole body dies. One of these, the rate of living theory, postulates that the faster the rate of an organism's oxygen basal metabolism, the shorter its life span (51). The rate of living theory of ageing, although helpful, is not entirely sufficient to

explain the maximum life span (52). Accumulation of cross-linked proteins damages cells and tissues, slowing down bodily processes resulting in ageing, as summarized in the cross-linking theory. One study shows that cross-linking reactions are involved in age-related changes in the studied proteins (53). Free radicals theory proposes that superoxide and other free radicals cause damage to the macromolecular components of the cell, giving rise to accumulated damage, causing cells, and eventually organs, to stop functioning (54). It has been shown that reactive oxygen species (ROS) signaling is probably the most important enzyme/gene pathway responsible for the development of cell senescence and organismal ageing and that ROS signaling may be considered a further development of the free radical theory of ageing (55). Somatic deoxyribonucleic acid (DNA) damage theory proposes that DNA damage occurs continuously in cells of living organisms. While most of this damage is repaired, sometimes it accumulates, as the DNA polymerases and other repair mechanisms cannot correct defects as fast as they are apparently produced. Genetic mutations occur and accumulate with increasing age, causing cells to deteriorate and malfunction. Therefore, ageing results from damage to the genetic integrity of the body's cells (56).

Programmed theories of ageing

The programmed theory has three sub-categories: programmed longevity theory proposes that ageing is the result of a sequential switching on and off of certain genes, with senescence being defined as the time when age-associated deficits are manifested (57); endocrine theory suggests that the biological clock acts through hormones to control the speed of ageing. Ageing is hormonally regulated and the evolutionarily conserved insulin-like growth factor-1 (IGF-1) signaling pathway plays a key role in the hormonal regulation of ageing (58). Finally, according to the immunological theory of ageing, the immune system is programmed to decline over time, which leads to increased vulnerability to infectious disease and, in turn, ageing and death. It is well documented that the effectiveness of the immune system peaks at puberty and gradually declines thereafter with advance in age (59). For example, as an individual grows older, their antibodies lose their effectiveness and fewer new diseases can be effectively combatted by the body, which causes cellular stress and, eventually, death (59). The relative number of cells undergoing programmed cell death (apoptosis) increases in people aged ≥ 80 (60) and the functions of the immune system deteriorate (61). An age-related decrease occurs both in the absolute number and in the percentage of peripheral blood T cells. Ferguson et al. reported changes in Ig's and a

slower rise and earlier decline of the antibody response with ageing (62). They also found that inverted CD^4+ T cells/ CD^8+ T cells ratio was associated with immune risk profile. Strindhall et al. found poor T-cell proliferative responses, high CD^8+ T cells and low CD^4+ T cells percentages for non survivors compared to survivors with 6-year follow-up, aged 86-94 years (63).

Inflammageing

Immunosenescence and immunogenetics have been studied from different angles (64), e.g. chronic infections have been studied from a histopathologic, molecular, epidemiologic and genetic view. One source concluded that the increased age in populations creates a “new burden on medical intervention as this increase is correlated with a higher prevalence of neoplasia and age-related diseases” (65).

Ageing affects both the innate and the adaptive immune system. With ageing, some well-adjusted regulatory systems do not act in their well-adjusted way any longer and the result is chronic low-grade production of proinflammatory molecules, with tissue injury as a consequence. Later, when a proper specific response is needed, the immune system is no longer able to act vigorously and precisely (12). For example, it is known that the risk to develop Alzheimer’s disease increases with ageing (66). As increased levels of proinflammatory cells with production of IL (e.g. IL-6 and IL-1 β) are associated with cognitive decline, it is suggested that this low-grade chronic inflammation, called “inflammageing”, could contribute to cognitive decline and Alzheimer’s disease (66). Inflammation could affect hippocampal neurogenesis and long-term potentiation, which correlate with the formation of memories (66). In cardiovascular disease (CVD), IL-6, IL-1 β and TNF- α have also been observed in the myocardium and peripheral tissues and seem to play an important role in the pathogenesis and progression of myocardial dysfunction (67). By measuring these proinflammatory cells in individuals with chronic heart failure, short- and long-term survival can be predicted (67). There is a correlation between ageing and development of cancer (68). More than 60% of new cancers and more than 70% of cancer deaths occur in individuals 65 years and older. Viral infections are known to be more common in the elderly, and some of them are able to directly induce tumorigenesis. Decline in anti-tumour immunity is proposed to be responsible for the increased incidence of cancer in the elderly (68).

Frailty

During the 1970s the heterogeneity among the elderly population became more widely recognized. The term “frail elderly” to describe a particular segment of the elderly population was coined by Charles F. Fahey and the Federal Council of Aging (FCA) in the US (69, 70). The term was not understood to apply to any specific group among the elderly. Others agreed that the characteristics of the frail elderly included “physical debilities and emotional impairment, as well as debilitating physical and social environments” (71). In 1976 the FCA stated about frail elderly people that “these persons require continuing support from society because of an accumulation of the debilities of increasing age” (72). In 1978 the FCA defined frail elderly people as “persons, usually but not always, over the age of 75, who because of accumulation of various problems often require one or several supportive services in order to cope with daily life” (71). In the early 1980s researchers began to define “frail” or “frail elderly” in their papers (73). Early definitions of “frail elderly” included: those aged 75 or more; a population of seniors who are particularly vulnerable because of mental impairment; older individuals admitted to a geriatric programme; those requiring institutional care; and seniors who are dependent on others for activities of daily living (ADLs) (73).

Frailty can be seen as a consequence of age-related multifactorial deterioration – physical, cognitive and sensory – resulting in vulnerability and lack of adaptability to internal stressors such as infection or new medication and/or external stressors such as fall at home (74, 75). When exposed to environmental challenges, frail individuals are at an increased risk of needing support, i.e. nursing home care, around the clock, and of hospitalization and mortality, compared with healthy older adults (76, 77).

Epidemiological data suggest that frailty is a transitional state and a dynamic process (74). The process starts with being non-frail, i.e. with no physiological or cognitive deficits, and continues with being pre-frail, i.e. with presence of one or two physiological or cognitive deficits. This condition identifies a subset of older people at high risk of progressing to frailty (75). Studies characterizing frailty conditions in this way typically adopt Fried et al.’s (2001) frailty criteria. A standardized definition of “frailty” that is more common, is that it is a clinical syndrome in which three or more of five criteria are present: low grip strength, low energy, slow walk speed, impaired physical activity, and unintentional weight loss (75). Another, more simple definition is

linked to the person's ability to perform ADLs, and cognitive function (tested using the Mini Mental State Examination (MMSE)) (78). Some researchers emphasize the importance of history taking and clinical examination in identifying frailty (79) and argue that greater importance must be attached to functional disability and cognitive impairment (80). Yet another approach consider that summing of deficits in health is another way to define frailty, on the grounds that the more deficits a person have, the more likely that person is to be frail (81). These deficits can be symptoms, signs, diseases, disabilities, or laboratory, radiographic or electrocardiographic abnormalities. This index, named the "frailty index (FI)", is often expressed as a ratio of present deficits to the total number of deficits considered and the results indicate strong validity (79, 82).

Table 2 illustrates how "frailty" has become a concept of interest in clinical assessment of elderly individuals. Since the 1970s, when the term "frail elderly" was first used, the interest has increased over the decades, from only a few publications to now nearly 6,000 (Table 2). As consensus about the definition of "frail" and "frailty" is missing, both nationally and internationally, the question arises whether different definitions of "frailty" affect the interpretation and reference intervals of analytes when comparing different groups of elderly.

Table 2: Number of publications in PubMed, found using the MeSH term "frail elderly", by decade.

Years	Number of publications
1979 or earlier	5
1980–1990	218
1991–2000	2,075
2001–2010	3,531
2011–2018 (7 years)	5,713

Analytes studied in the present thesis

Immunological markers

Approximately 15–20% of all Ig in blood is IgA. Immunoglobulin A antibody plays an important role in the defence against bacteria outside the mucous membrane as antibodies bind together and block bacterial binding to the epithelium. Of all Ig in the blood, 70–75% is IgG. This consists of four subgroups, IgG1–4, among which deficiency of IgG2 can cause serious infections. A deficiency in IgM is rare, but can lead to infection problems of different degrees of difficulty. A slight increase in IgM can be seen in many viral infections, for example hepatitis A, mononucleosis, rubella, cytomegalovirus and Coxsackievirus infection (83).

Another immunological marker is C3, which is the quantitatively dominating protein among the complement (C) factors. Low levels in plasma are related to the first appearance of acute glomerulonephritis and to immune complex diseases. Increased levels of C3 indicate inflammatory activity without increased complement consumption. Like C3, C4 is an acute-phase protein whose synthesis increases within a few days in infection (84).

Interleukins (IL) analysed in the present study were IL-1 β , IL-1 receptor antagonist (IL-1RA), IL-6, IL-8 and IL-10 (Table 3). Of these, IL-1 β plays an important role in the inflammatory response of the body against infection, among other things through response to lipopolysaccharides in the walls of gram-negative bacteria, which also induces elevated body temperature (31). The IL-1RA competes with IL-1 β , among others, for receptor binding, counteracting its role in immune activation. Another IL investigated, IL-6, is produced by cells in the innate immune system such as DCs, monocytes, B cells and subsets of activated T cells. It causes synthesis of proteins, such as fibrinogen, which contribute to the acute-phase response, i.e. inducing acute-phase proteins such as C-reactive protein (CRP). An IL that both induces chemotaxis in granulocytes, causing them to migrate towards the site of infection, and induces phagocytosis is IL-8 (85). Because neutrophils are initial players in acute inflammation, IL-8 and other ILs are consistently among the first markers to be expressed and released by the various cell types involved in inflammation. Lastly, IL-10 is known as a multifunctional cytokine that inhibits activation and effector function of T cells, monocytes, and macrophages (31).

Clinical chemistry markers

Among the clinical chemistry markers we investigated, aminotransferases occur in many tissues, but the highest concentrations of aspartate aminotransferase (AST) occur in the heart, liver and the muscles of the skeleton. The main activity of alanine aminotransferase (ALT) is in the liver (86). Albumin is responsible for 80% of the colloid osmotic pressure and the distribution of water between the plasma and the intercellular room. Albumin is an important transport protein for fatty acid from the layer of fat to the liver, the muscles of the skeleton, and the transport of bilirubin to the hepatocytes. One-third of all calcium in body is bound to albumin. Also, many medications bind to albumin (87). Another marker of liver function is gamma-glutamyltransferase (γ -GT). This enzyme is important for the transport of amino acids into the cell, foremost in the kidneys, the prostate, pancreas and liver (86). Hydrolysis of some peptides is catalysed by γ -GT enzymes. The chemistry marker creatinine constitutes the anhydrid form of creatine which in relaxed muscles is stored as creatine phosphate. Creatine is released at muscle contractions, and one part is converted to creatinine, which means that the creatinine level is dependent on muscle mass. The secretion of creatinine occurs through the kidneys (88).

Other important biomarkers we investigated are lactate dehydrogenase (LDH), phosphate and sodium. All somatic cells contain LDH in the cytoplasm. In pathological changes with increased cell permeability, levels of LDH increase in blood (86). All phosphate in the body enters as phosphate ions in bone mineral and phosphorus is involved in practically all metabolic processes of the body (89). Sodium is absorbed by nutrients in the renal tubules and determines the osmolality of the body fluids. Minor parts are lost through the skin, but at high perspiration the loss of sodium can be considerable. Reduced steroid production, treatment with aldosterone inhibitor and diseases can cause impaired function of the renal tubules and reduce the reabsorption, which can lead to a negative sodium balance (87). Lastly, we looked at urea of which there normally is a large range in serum depending on ingestion of dietary proteins (89).

Aims of the thesis

Overall aim

An overarching aim of the thesis was to interpret and assess circulating levels of some clinical laboratory analytes in relation to conventional reference values in ≥ 80 -year-old, “apparently healthy”, “moderately healthy”, and “frail” individuals.

Specific aims

Paper I

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.

Paper II

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.

Paper III

To study whether 1-year changes in complete blood count (CBC) (including Hb, RBC, erythrocyte volume fraction (EVF), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), WBCs and PLT), CRP and ILs (including IL-1 β , IL-1RA, IL-6, IL-8 and IL-10) are associated with 8-year survival in elderly NHRs, aged ≥ 80 years.

Paper IV

To investigate the effect the classification into healthy, moderately healthy and frail, based on Activities of Daily Living (ADLs) and Mini Mental State Examination (MMSE) or Frailty Index (FI) scores, on the interpretation of the laboratory results regarding: albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and gamma- glutamyltransferase (γ -GT) levels.

Methods

Study populations

Data originated from other studies (3, 41, 90, 91), in which blood samples were collected. Some blood samples were analysed soon after the blood sampling, while others were frozen until analysis. Some analyses were performed within the original study and some were conducted by the author M.E. of this thesis (Table 3).

The NHR 2000 study (Paper I)

Nursing home residents (n=138) 80–99 years of age gave informed consent for blood sampling and were included in the present study. Mean age was 86.8 years; 66% were women and about 22% had multiple disease conditions. Data on chronic diseases were collected from the medical records. Almost half (42.8%) of the NHRs received paracetamol >3 g/day and 15.2% were malnourished (Table 4). Only nine (6.5%) out of 138, although aged, were assessed as “healthy”, in terms of being free from heart disease, autoimmune disease, dementia, stroke, diabetes mellitus type 2, and malnutrition and/or not receiving daily paracetamol. All of them needed daily care and support, assessed using the ADLs Staircase, based on the Katz index of independence in ADLs (92), and all lived in special housing for elderly people. The Katz ADL index includes six categories of personal ADLs (PADLs): bathing, dressing, toileting, transfer, continence, and feeding, and four categories of instrumental ADLs (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 (93, 94). Data were also collected regarding MMSE consists of 21 questions testing memory, naming, orientation, attention and constructive ability (95). The maximum score is 30 and a score of <27 indicates cognitive impairment.

Blood donors (Paper I)

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 aged 18–64 years. The values for C3 and C4 were based on 123 blood donors, 22–63 years old, mean age 41.0; 19.5% of whom were women. For the donors used (n=189) for analysis of IgA, IgG and IgM, age and gender were unknown, but it can be assumed that the sample was probably similar to the sample of 123 blood donors.

The NORIP raw origin (Paper I) and NORIP raw origin 80 (Papers I, II and IV) studies

The total database of the Nordic Reference Interval Project (NORIP) study (41) was used with original data, named “NORIP raw origin”. Blood samples from the total NORIP raw origin population included 2,777 individuals 18–90 years (mean 46.6 years) old, 53% of whom were women (Figure 2). Inclusion criteria were as follows: being subjectively healthy; not being pregnant or breastfeeding; not having been seriously ill during the past month; not having consumed more than 24 g pure alcohol in the last 24 hours; not having given blood as a donor in the past 5 months; not having taken prescribed drugs other than oral contraceptives or oestrogens during the past 2 weeks; not having smoked during the hour before blood sampling. Within NORIP study, neither MMSE scores nor ADLs were measured, but the study excluded neither cognitively nor physically impaired persons.

Original data on individuals ≥ 80 years old, referred to as the “NORIP raw origin 80” cohort, from the NORIP study (41) were included in Papers I, II and IV. This population included 64 individuals 80–90 years (mean 81.9 years) old, 50% of whom were women.

Figure 2: Age and country distribution of all individuals (n=2,777) included in the Nordic Reference Interval Project (NORIP) study (41). Figure reproduced with permission from Pål Rustad, Oslo, Norway.

The NHR 2008 study (Papers II, III and IV)

Nursing home residents (n=168) aged 80–101 years (mean age 88), 75% of whom were women, were included in an investigation during 2007–2009 in two municipalities in the south of Sweden (3). All residents needed daily care and support, but 6% managed PADLs with minor assistance. The NHRs all lived in group housing for the elderly (see “The NHR 2000 study” above, for details about physical and cognitive measures for this sample). Data on chronic diseases were collected from the medical records. Dementia was diagnosed in 61.3%, diabetes mellitus type 2 in 18.5%, heart disease in 59.5%, malignancy in 25% (data not shown) and stroke in 34.5% (Table 4).

In Paper III, during a 1-year follow-up, the NHRs were carefully monitored by nursing staff for suspected infection and signs of medical, physical or cognitive change, which could be an expression of infection, as described elsewhere (3). In addition, blood sampling was done at baseline, and after 6 and 12 months, i.e. when the NHRs were stable in their disease status and habitual condition. Each blood sampling was separated by at least 2 weeks from occasions of suspected infection. Owing to mortality and missed sampling, the number of individuals with available blood samples had decreased by the 6 and 12-month follow-up (Table 5). Dates of death were collected from the National Death Register 8 years after baseline. At the end of the follow-up period, ten individuals were alive.

Table 5: Number of blood samples collected at baseline (inclusion) and at the 6 and 12-month follow-up, with reasons given for unavailable samples (death or missed sampling) (Paper III).

Analyte	Baseline	Death	Missed sampling	6 months	Death	Missed sampling	12 months	Baseline and 6 months	Baseline and 12 months	Baseline, 6 and 12 months
	n	n	n	n	n	n	n	n	n	n
CBC	165	29	11	125	14	6	105	124	104	102
CRP	165	29	11	125	14	6	105	125	105	103
ILs	152	29	8	115	14	11	90	104	81	69

CBC = complete blood count; CRP = C-reactive protein; ILs = interleukins.

Elderly in Linköping Screening Assessment (ELSA 85) (Papers II and IV)

The Elderly in Linköping Screening Assessment (ELSA 85) cohort consisted of individuals (n=338) aged 85 years, 57% of whom were women and about 20% of whom had multiple disease conditions. Almost 60% of the ELSA 85 cohort were on daily painkillers and 10.1% were malnourished (Table 4). Physical and cognitive status based on PADLs, IADLs (93, 96) and MMSE scores (95) was collected from the medical records and questionnaires administered at an introductory visit. Assessment of PADLs was made based on the items “manages without assistance”, “needs some assistance” and “needs 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 small-scale shopping, do large-scale shopping, and clean and wash, with the ability levels “no difficulty”, “some difficulties”, “difficult” and “too difficult” (96).

Table 4: Distribution of chronic diseases and prescribed analgesic drugs in the NHR 2000, NHR 2008 and ELSA 85 cohorts. More than one disease could occur in a participant (3, 90, 91).

Condition	NHR 2000 n=138 (%)	NHR 2008 n=168 (%)	ELSA 85 n=338 (%)
Women	91 (66)	126 (75)	193 (57)
Chronic heart disease	95 (68.8)	100 (59.5)	60 (17.8)
Dementia	39 (28.2)	103 (61.3)	14 (4.1)
Stroke	33 (23.9)	58 (34.5)	51 (15.1)
Diabetes mellitus type 2	23 (16.7)	31 (18.5)	62 (18.3)
Kidney disease	1 (0.7)	11 (6.5)	2 (0.6)
Liver disease	1 (0.7)	1 (0.6)	3 (0.9)
Autoimmune disease	7 (5.1)	23 (13.7)	15 (4.4)
COPD	5 (3.6)	8 (4.8)	60 (17.8)
Suspected or confirmed malnutrition	21 (15.2)	75 (44.6)	34 (10.1)
Daily painkillers	59 (42.8)	88 (52.4)	202 (59.8)

COPD = chronic obstructive pulmonary disease.

Laboratory sampling and analysis

The NHR 2000 study (Paper I)

During 2000–2001 venous blood samples, taken between 08:00 and 11:00 in the morning, were collected in ethylene diamine tetra-acetic acid (EDTA) tubes, centrifuged and frozen at -70°C until analysed. Analytes studied were selected based on their suitability for EDTA analysis. The following were analysed, using routine methods, by personnel at the Division of Laboratory Medicine, Ryhov Hospital, County Council of Jönköping, during 2000–2001: IgA, IgG, IgM, C3, C4, ALT, albumin, AST, creatinine, γ -GT, LDH, phosphate, sodium and urea (Table 3).

Blood donors (Paper I)

Venous blood was collected during 1989–1990 in EDTA tubes, centrifuged and frozen at -70°C until analysed. Plasma was analysed, using by accredited routine methods by personnel at the Clinical Chemistry, Diagnostic Center, Linköping. The Beckman Coulter Immage 800 Nephelometry Analyzer (Beckman Coulter Instruments, Inc., Fullerton, CA, USA) was used for measurement of immunology parameters (Table 3).

The NORIP raw origin (Paper I) and NORIP raw origin 80 (Papers I, II and IV) studies

Venous blood was collected during 2000–2001 from at least 25 reference individuals evenly distributed for gender and age, along with five controls, by each of 102 Nordic laboratories and measured using their local system. Tubes without anticoagulant were used for serum, and tubes using lithium heparin as anticoagulant were used for plasma, and centrifuged. Some of the samples were collected for analysis at the laboratory included in the NORIP study and some were sent for analysis to a central biobank run by the Danish Institute for External Quality Assurance for Laboratories in Health Care (DEKS). Samples were stored in three -80°C freezers, all located at Herlev University Hospital, Herlev, Copenhagen, Denmark (97). The plasma and serum were transferred into secondary tubes and frozen at the participating laboratories at -80°C until analysis in 2000–2001. For the enzymes, only results obtained by routine assay conditions at 37°C , which were compatible with and traceable to the IFCC reference methods, were accepted for use, in line with enzyme methodology. To make comparisons between the different laboratories possible, each laboratory was sent control materials with instructions on how to handle the samples. Først Medical Laboratory in Oslo, Norway, is the central database with all collected data. Background information on the participants in the NORIP study, together with levels of

analytes, was obtained from Pål Rustad, Først Medical Laboratory, Oslo, Norway, with permission for research use in this thesis.

The NHR 2008 study (Papers II, III and IV)

In 2007–2009, venous blood samples were collected in the morning between 08:00 and 11:00 in evacuated tubes, using EDTA as anticoagulant (Papers II, III and IV). The plasma was transferred into secondary tubes and frozen to -80°C until analysis. Some tubes were analysed by the personnel at a laboratory in Eskilstuna in 2015. Analyses were conducted using an automated analyser (Siemens ADVIA 1800; Siemens Healthcare Diagnostics, Inc., Tokyo, Japan). Methods used were bromocresol purple (BCP) for albumin, and enzymatic creatinine methods and the IFCC reference measurement procedure at 37°C for ALT, AST and γ -GT. For Paper III, CBC and CRP were measured, using routine methods, by personnel at primary health care laboratories in Eksjö and Boxholm, Sweden. Methods employed in Eksjö were optical light scatter and colorimetric determination for CBC using a Cell Dyn 3200 analyzer (Abbott Diagnostics, St Clara, CA, USA) and immunoassay with ADVIA 1200 (Siemens Healthcare Diagnostics, Inc., Tokyo, Japan) for CRP. In Boxholm, a Swelab Alfa Plus analyser (Boule Diagnostics AB, Spånga, Sweden) was used for CBC and CRP, applying impedance technique and photometric determination as methodology. The cytokines IL-1 β , IL-1RA, IL-6, IL-8 and IL-10 were analysed during 2011 by the author of the present thesis (M.E.) at the Division of Laboratory Medicine, Ryhov Hospital, Jönköping, Sweden, using Luminex (Bio-Rad Laboratories, Hercules, CA) (Table 3). Luminex technology is based on microspheres that are distinguishable by fluorescence (98). The different coloured beads can be coated with different antibodies specific for a particular protein. The beads are mixed with the sample and the protein is bound to the beads. An antibody labelled with phycoerythrin attaches to the bound protein. Phycoerythrin and the mixture in the beads are illuminated by laser and the fluorescence is detected. The colour of the beads identifies the protein and the fluorescence from phycoerythrin is proportional to the amount of the protein in the sample (98).

The ELSA 85 study (Papers II and IV)

Venous blood was collected during 2007–2008 in tubes using lithium heparin as anticoagulant, and centrifuged. The plasma was transferred into secondary tubes and kept at -80°C until analysis, using an automated analyser (Siemens ADVIA 1800; Siemens Healthcare Diagnostics, Inc., Tokyo, Japan), and BCP for albumin and Jaffé's method for creatinine, by the personnel at the Clinical Chemistry, Diagnostic Center, Linköping. Analyses of ALT, AST and γ -GT were performed in 2015 by the author of this thesis (M.E.), using Selectra ProM (ELITechGroup, Puteaux, France) (Table 3). Methods used were routine assay conditions at 37°C , which is compatible with and traceable to the IFCC reference methods.

Table 3: Distribution of study populations and analytes investigated in the different papers. Analyses marked with X have been performed by the personnel at the clinical chemistry laboratories and ♦ were performed by the author of this thesis (M.E.).

		Paper I	Paper II	Paper III	Paper IV
Cohorts	NHR 2000 (n=138)	X			
	Blood donors' C3 and C4 levels (n=123)	X			
	Blood donors' IgA, IgG and IgM (n=189);	X			
	NORIP raw origin (n=2,777)	X			
	NORIP raw origin 80 (n=63)	X	X		X
	NHR 2008 (n=168)		X	X	X
	ELSA 85 (n=338)		X		X
Analytes	Albumin	X	X		X
	ALT	X	♦		♦
	AST	X	♦		♦
	C3, C4	X			
	CBC			X	
	Creatinine	X	X		X
	γ -GT	X	♦		♦
	IgA, IgG, IgM	X		X	
	IL-1 β , IL-1RA, IL-6,			♦	
	IL-8 and IL-10			♦	
	LDH	X			
	Phosphate	X			
	Sodium	X			
	Urea	X			

See: List of abbreviations

Statistics

Statistical analysis was performed using PASW Statistics software (SPSS Inc., Chicago, IL, USA), version 20 for Paper I, version 24 for Paper II and version 25 for Papers III and IV. The level of significance was set to $p < 0.05$. Table 6 presents the distribution of different statistical tests used in the different papers.

Paper I

Student's *t*-test was used to compare mean values between the NHR 2000 cohort and the blood donors, and the NORIP raw origin and NORIP raw origin 80 cohorts for the investigated analytes; C3, C4, IgA, IgG, IgM, albumin, ALT, AST, creatinine, γ -GT, LDH, sodium, phosphate and urea. Student's *t*-test was also used to compare mean values for subgroups within the NHR 2000 population, i.e. groups with or without: medication, malnutrition, and different levels of physical and cognitive status or presence of heart disease, autoimmune disease or chronic obstructive pulmonary disease (COPD).

Descriptive statistics were constructed and illustrated in box plots. Medians and the range between the 25th and 75th percentiles were presented in the box, with whiskers indicating 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.

Paper II

Pearson's and Spearman's correlations were used to determine correlations between analytes and independent variables: age, gender, height, weight, smoking status; dementia, asthma, presence of 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 ADL and MMSE scores. Analysis of variance (ANOVA) and Tukey's *post hoc* test were used to compare mean differences between the different cohorts, "healthy", "moderately healthy" and "frail". Predictive factors for change in levels of analytes were examined with linear regression and the independent variables. The distribution of albumin, ALT, AST, creatinine and γ -GT was presented in box plots, stratified by gender and into "healthy", "moderately healthy" and "frail".

Paper III

For comparisons of mean levels of analytes at baseline and at 6- and 12-month follow-up, Student’s *t*- test was used. For statistical significance with Bonferroni correction, an adjusted *p*-value of 0.0167 was required when comparing the three measuring points. To explore predictors of survival, the Cox regression model with segmented time-dependent covariates was used. Survival, in days from the inclusion date, up to a maximum of 8 years, was the dependent variable, with levels of analytes, age and gender as independent variables. Cox regression with a segmented time-dependent covariate was used to adjust for the samples collected at baseline and at 6 and 12 months. To be included in this Cox analyses, the individual had to have survived >6 months and analytes from at least two of the three consecutive measuring occasions were needed. Five-year age intervals were used: 80–84 years, 85–89 years, 90–94 years and 95–101 years. The group of individuals aged 80–84 years were considered as a reference for the other age groups.

Paper IV

For statistical comparisons of mean analyte values, the individuals were randomly divided into two groups and then classified as “apparently healthy”, “moderately healthy” or “frail”. Student’s *t*-test was used to compare mean analyte values between the groups. Descriptive statistics were constructed to present the distribution of “apparently healthy”, “moderately healthy” and “frail2 based on ADL and MMSE scores or the number of deficits, using the FI. Descriptive statistics were also constructed using medians and 25th to 75th percentiles and presented in box plots with whiskers indicating minimums and maximums. Further, 2.5th and 97.5th percentiles were calculated, representing lower and upper limits for the analytes in the different groups.

Table 6: Statistical tests used in the different papers.

	Paper I	Paper II	Paper III	Paper IV
Student’s <i>t</i> -test	X		X	X
Pearson’s and Spearman’s correlations		X		
Analysis of variance (ANOVA)		X		
Tukey’s <i>post hoc</i> test		X		
Linear regression		X		
Bonferroni correction			X	
Cox regression model with segmented time-dependent covariates			X	
2.5th and 97.5th percentiles				X

Ethics

All the participants or their next of kin gave both oral and written informed consent for participation and for storage of blood samples for later analysis in the investigations. It was made clear that participation was voluntary and could be withdrawn at any time and that only de-personalized data would be stored in a computer. The Regional Ethics Board in Linköping, Sweden, Dnr: 99017 (Paper I), 141-06 (Papers II and IV) and M-8206 (Papers II, III and IV), and the health service directors of community care and geriatric staff nurses gave their permission to conduct the study (Papers I and II). Blood donors gave informed consent to provide extra blood for the establishment of reference intervals (Paper I). Ethics committees in the five Nordic countries approved the NORIP study (Papers I, II and IV).

Results

To investigate whether reference intervals provided for laboratory analytes are valid for frail elderly individuals we conducted the study reported in Paper I. Levels of some analytes were compared between 138 individuals ≥ 80 years old living in nursing homes, named the NHR 2000 study population, and reference populations. For the immunological analytes (i.e. IgA, IgG and IgM), the reference population consisted of two groups of blood donors. For the chemical analytes (i.e. albumin, ALT, AST, creatinine, γ -GT, LDH, sodium, phosphate and urea), the reference population were from the NORIP raw origin. The results indicated differences for some of the analytes, but not all. Complement factor 3 ($p < 0.001$), C4 ($p < 0.001$) and IgG levels ($p < 0.05$) were higher, while IgM levels ($p < 0.001$) were lower in the NHR 2000 population compared with reference blood donors. Levels of ALT ($p < 0.001$), phosphate ($p < 0.001$), albumin ($p < 0.05$) and sodium ($p < 0.01$) were lower, while creatinine and urea levels were higher ($p < 0.001$) in the NHR 2000 population compared with the NORIP raw origin cohort. It was unclear whether the differences were due to differences in age between the elderly and the reference populations or whether the elderly individuals had chronic diseases and were on medication. This could not be explained by the study and initiated further investigation (Paper II).

The study reported in Paper II included 569 elderly individuals ≥ 80 years old. The study populations were the NORIP raw origin 80, ELSA 85 and NHR 2008 cohorts. The individuals were divided into three groups depending on health status: “apparently healthy”, “moderately healthy”, and “frail”, based on diseases, medications and physical and cognitive abilities (i.e. ADL and MMSE scores). Statistical differences between the groups were found for the investigated analytes albumin, ALT, AST, creatinine and γ -GT. “Frail” individuals had lower albumin and ALT and higher γ -GT than “healthy” and “moderately healthy” individuals ($p < 0.01$). “Moderately healthy” elderly people had lower AST and higher creatinine than “frail” and “healthy” elderly people ($p < 0.01$). However, as consensus on the meaning of “frail” was missing, a further study was needed (see Paper IV).

In Paper IV, 569 elderly individuals aged 80 years and older were divided into “apparently healthy”, “moderately healthy”, and “frail” using two different classification models, one based on ADL/MMSE and the other being the FI. The study populations were the NORIP raw origin 80, ELSA 85 and NHR 2008 cohorts. The elderly individuals were randomly divided into two groups. One was subdivided into “apparently healthy”, “moderately healthy” and “frail” based on physical and cognitive abilities by ADL/MMSE and the other group was divided based on the FI. There was no statistical difference found between “apparently

healthy” and “moderately healthy” groups, regardless of classification model used. Among “frail” individuals, differences in levels occurred for three out of the five investigated analytes: ALT, creatinine and γ -GT ($p<0.05$), with lower levels occurring when the FI classification model was used.

To summarize Papers I, II and IV, levels of ALT and creatinine from the NORIP raw origin cohort, together with the NHR 2008 and ELSA 85 cohorts, divided into “healthy”, “moderately healthy” and “frail” (based on ADL/MMSE or FI scores), are presented in Figure 3. The box plot in the Figure presents reference intervals for the analytes, as proposed in the NORIP study (41).

Figure 3 A–B: Box plots summarizing results from Papers I, II and IV. Distribution of alanine aminotransferase (ALT) and creatinine levels in the NORIP raw origin cohort compared with the distribution across the three sub-cohorts, of healthy, moderately healthy, and frail elderly individuals, with classifications based on activity of daily living (ADL) and mini mental state examination (MMSE) or Frailty Index (FI) scores. Medians and variation between the 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. Vertical lines present the reference intervals proposed by NORIP study (41).

The aim of Paper III was to study whether 1-year changes in CBC (including haemoglobin (Hb), red blood cell (RBC), EVF, MCV, MCHC, white blood cell (WBC) and platelet count (PLT)), CRP and IL-1 β , IL-1RA, IL-6, IL-8 and IL-10 are associated with survival in elderly NHRs aged ≥ 80 years. The NHR 2008 study population were followed for 1 year, with the aim to validate the Early Detection of Infection Scale (EDIS) (4). The most prominent results were that elevated levels of CRP and IL-8 during 1-year follow-up were associated with reduced length of survival in elderly NHRs. For Hb, the mean value was lower at 12 months compared with 6 months ($p < 0.01$). Regarding RBC, mean values at 12 months were lower than at baseline ($p < 0.01$). Mean corpuscular Hb concentration ($p < 0.001$) was lower at 12 months compared with the 6 months' follow-up, and MCV was higher at 12 months compared with baseline ($p < 0.01$). Interleukin-1 β showed higher levels at 12 months ($p < 0.001$) compared with baseline and 6 months

General discussion

The insufficient knowledge regarding natural ageing and/or how it affects the levels of laboratory analytes is a strong argument for studying at least the most common analytes in relation to health and disease in older individuals. Age is accompanied by a general decline in organ functions, in particular cardiovascular, pulmonary and kidney functions (99). It is difficult to determine whether the change is ascribable to age alone, independent of environmental or disease processes. In addition, infectious disease in elderly individuals is often presented with nonspecific signs and symptoms while specific ones are missing (4). Hence, analytes are important as a basis for investigation when the caregiver confirms or denies the presence of both chronic and infectious disease. Whether “abnormal” laboratory values, i.e. values below or above the reference intervals, in an elderly person reflect disease processes, or whether they are related to physiological consequences of ageing is not always clear. The compilation of reference intervals for the elderly is complicated by numbers of factors, including the presence of multi-system disease, the effects of diet, malnutrition and the use of medication. Current reference values for common laboratory analytes are often based on measurements from apparently healthy persons aged 18–65 years (15). The prevailing view regarding laboratory reference intervals is that levels are derived from persons considered to be healthy; therefore the term “health-related” (or “health-associated”) reference values has been proposed (9). The present thesis emphasizes that this approach may cause problems when laboratory results from ≥ 80 -year-old individuals with chronic disease and on chronic medication use are being interpreted.

Reference intervals and elderly

Interpreting results of laboratory analytes in elderly individuals can be a complex task. The results from the present thesis show that current reference intervals proposed for some immunological and chemical analytes (41) are not valid for frail elderly individuals above 80 years, living in nursing homes. An increase in IgG, IgM, C3 and C4 in this age group indicates an activated immune system, which may be part of a chronic low-grade inflammation (100, 101). This could result in misleading and even dangerous assessments, as normal conditions may appear pathological (or *vice versa*), and thus lead to unnecessary treatment. As it is known that levels of IL-1 and IL-6 increase with ageing, and they in turn induce acute-phase proteins, our results seem reasonable (100).

Different procedures for developing reference intervals for elderly

Various attempts have been made to adjust reference intervals to older individuals' state of health (10, 40, 42-47). Dividing elderly individuals into "non-diseased" and "non-healthy" depending on how many times they seek medical care may seem reasonable, but is too wide and hence insecure to use in clinical practice (46). A question immediately raised is how to decide who is "non-diseased" and who is "non-healthy"? Survival over 6 years has been used to evaluate whether an individual is healthy or not (43) but that method, too, is not optimal in clinical settings. Both the need of seeking care and survival might be affected by correct medical treatment in spite of the presence of chronic disease. Is it possible how to know which patient will live another 6 years and which patient will not survive that many years? Comparing levels of analytes between different age groups has been investigated by several researchers (10, 40, 47). Unfortunately, the number of elderly individuals included in some studies is low with the oldest age groups are >60 years, n=82 (10), 76–80 years, n=20 (40) or >70 years, n=279 (47), respectively. As life expectancy have improved (1) these investigations still leave individuals ≥ 80 outside (10, 40, 47). Rigorous inclusion and exclusion criteria have been used, such as extensive neurological, clinical chemistry, psychological, genetic examinations (42), which means that the proposed reference intervals will not be applicable to a large proportion of elderly individuals. A further limitation when comparing results from other studies with samples from elderly individuals is that the description of inclusion criteria in some studies are vague (Table 1). Reference intervals provided by Huber et al. for apparently healthy 75-year-olds were considered applicable for younger individuals for some analytes (42), but not for most elderly ≥ 75 -year-olds as many of them have different diseases. Despite a huge Canadian project including almost 12,000 participants, and investigating 24 laboratory analytes, this project leaves a large group of people >80 years, without any reference intervals (44). Another study, investigating individuals 80 years and older, focused on diabetes, high glucose levels and CVD (45), but not on other diseases. For the analytes investigated in the present study (Paper II), CVD affected the levels of γ -GT but none of the other analytes investigated.

Others (10, 40, 42-47), have briefly discussed diagnosis, survival and age groups in the context of adjusting reference intervals to elderly individuals. In the present thesis, the significance of considering physical and cognitive decline in relation to reference values was focused from the start. The first study showed different levels, for some analytes but not all, for frail elderly people compared with NORIP raw origin (41). Later, the elderly population were therefore divided according to health status, thus taking more than their age into account. The first attempt to classify individuals by health status was made using ADLs as physical marker together with MMSE scores to determine cognitive ability. Furthermore, classification of individuals according to health status was done by counting deficits, using the FI.

Levels of albumin in elderly

Like others (10, 40, 44-46), we found decreased levels of albumin in elderly NHRs, compared to the reference population of disease- and medication-free individuals, i.e. NORIP raw origin. This result was further confirmed when lower levels of albumin were measured in elderly NHR men and women, both together and separately by gender. The reasons are probably malnutrition and/or ongoing inflammatory processes (102), but the lower levels may also be due to changed appetite in depression and dementia (103). For albumin, special reference intervals developed by the NORIP study apply to individuals >70 years old (41). The results in the present study show that the intervals fit elderly individuals quite well if they are classified as “healthy” or “moderately healthy”, which is in concordance with others (45). However, in “frail” individuals, irrespective of classification model (ADL/MMSE or FI) used, albumin levels were lower than in “healthy” and “moderately healthy” individuals and, moreover, they were lower than, and partly outside, the reference intervals proposed by NORIP study (41).

Levels of ALT, AST and γ -GT in elderly

The present results show that frail NHR have lower levels of ALT compared with healthy reference populations. Also, “frail” elderly individuals had lower levels than “moderately healthy” and “healthy” men and women of the same age, ≥ 80 years. Furthermore, individuals classified as “frail” based on their FI score had lower ALT levels compared with “frail” individuals classified on the basis of their ADL and MMSE scores. For AST and γ -GT, no differences were seen between frail NHRs and both of the reference populations, blood donors and NORIP raw origin. Elevated levels of ALT are known to occur in liver injury, as with an overdose of paracetamol, or in heart injuries or pulmonary embolism affecting the liver. Both the NORIP study (41) and the Canadian Health Measure Survey (CHMS) (44) suggest higher reference intervals for ALT, AST and γ -GT than

found in our study populations. Levels of AST increase with damage to heart or skeletal muscles and to the liver (84). Also, regarding γ -GT, consumption of some medications or alcohol can raise the levels. Some researchers (10) have found higher γ -GT levels in middle-aged individuals >50 years, compared with younger individuals 20-50 years, and some have reported higher AST levels in elderly women, 80-year-old, compared with younger women, >18 years (44, 45). In the present study after classification based on health status, “moderately healthy” individuals showed lower levels of AST and γ -GT compared with “frail” elderly individuals.

Interpretation of levels of ALT, AST and γ -GT in elderly

These results in the present thesis indicate the importance of taking health status into account when interpreting levels of ALT, AST and γ -GT in individuals aged 80 and over. The ALT, AST and γ -GT levels, regardless of health status (healthy, moderately healthy or frail) were at the lower end, but within the reference intervals proposed by the NORIP study (41). Therefore, clinically it may be more important to be extra observant of elderly people who have elevated levels of ALT, AST and γ -GT, even if they are still within the reference intervals for these analytes. The results indicate the importance of using the classification of “frail” to identify these individuals as some of their analyte levels are much lower than the proposed reference intervals. Further on it is essential that consensus about the definition of “frail” is reached. Reference intervals that take only age and not also health status into account indicate that this is not satisfactory.

Creatinine levels in elderly

Concerning creatinine, we found that frail elderly NHRs had higher levels compared with the elderly people classified as healthy. Interestingly, the “moderately healthy” group in the present study had the highest creatinine levels, regardless of classification model (ADL/MMSE or FI scores). A plausible reason for this might be that they were receiving good medical care and were able, despite chronic disease, to still manage themselves in daily life. The present results show that 25% of increased creatinine levels in the elderly were associated with kidney disease, but there was an association also with COPD, male gender and a high MMSE score. Others have found higher creatinine levels in middle-aged and elderly: >50 years (10) and 80-year-old (45), compared with younger individuals: 20-50 years (10) and >18 years (45) – however, without taking into account their state of health. In general, kidney function is known to deteriorate with ageing (99). Another factor that can impair renal function, especially in frail elderly people, is decreased muscle mass (99), which can be a consequence of poor nutritional intake and reduced mobility, common conditions in NHRs.

Early detection of infectious disease when diffuse symptoms

Interestingly, we found in line with others (104) that elevated levels of CRP and IL-8 at baseline when included in the study, were significantly associated with shorter survival, which probably is related to inflammatory processes (67). Many diseases common in elderly people, such as Alzheimer's disease, CVD and cancer, are often associated with inflammatory processes (66-68). It is well known that infectious diseases in frail elderly people are challenging to detect because of lack of specific signs and symptoms (4, 105, 106). Measuring CRP with a bedside instrument could be an option in the nursing home setting to detect suspected infection early on in this population. Bedside instruments have been developed to be user-friendly; however, training to use them is needed, otherwise the results might be inappropriate.

Health status in elderly

From the present thesis it is obvious that there is a need for reference intervals that consider age together with health status in elderly individuals. Moreover, there were substantial differences in health status between the included individuals in the present investigations, reflecting the need for individual evaluation. As seen in the present thesis, when developing reference intervals and studying elderly individuals, there is a high risk that moderately healthy or frail elderly people are excluded while an elite of the healthiest individuals remains (107). However, ordinary senior reference populations without any diseases, medication and supplementation are too rare and cannot be regarded as representative of the entire elderly population (108). In the present study (Paper I) only nine (7%) out of 138 NHRs were assessed as "healthy" in terms of being free from heart disease, autoimmune disease, dementia, stroke, diabetes mellitus type 2 and malnutrition, and not taking daily painkillers. Interestingly, these healthy individuals did not show any difference in levels of the investigated laboratory analytes compared with the NORIP raw origin or NORIP raw origin 80 cohorts. This may indicate that changed levels of some laboratory analytes are due to presence of disease or medication rather than to ageing.

Within the present thesis, reference intervals were calculated in a similar fashion as described in NORIP study and by Helmersson-Karlqvist et al. (41, 45). As an attempt to explore and understand the consequences of different approaches related to the concept "frail elderly", we also divided an elderly cohort into "healthy", "moderately healthy", and "frail" on the basis of different classification models (ADL/MMSE versus FI score). The reference intervals provided by the NORIP study and Helmersson-Karlqvist et al. seem to be appropriate for the "healthy" and "moderately healthy", but not for the group of "frail2 elderly individuals, regardless of classification model (ADL/MMSE or FI). One probable

reason for this is that the NORIP study only included healthy individuals and in the study by Helmersson-Karlqvist et al., only high levels of glucose and CVD were taken into consideration (41, 45). This is well in line with how frail elderly were seen when the concept began to be used in the 1970s, when it was stated that frail elderly people constitute a particular segment of the elderly population (69, 70). Further studies are needed, however, and our findings suggest that health status should be taken into consideration when developing reference intervals for elderly people, i.e. individuals >80 years old.

Interpretation of laboratory analyte results

There are many reasons for ordering a laboratory test; this is not always done to decide whether somebody is healthy or sick, but also to follow up a patient and to observe whether the value has changed spontaneously or as a result of therapy (109). Reference intervals are provided from the laboratories to facilitate the interpretations of analyte results, for every specific individual. Laboratories need to be clear about what reference sample have been used, i.e. what individuals have been included in the production of the reference intervals. Were they free from diseases and/or medications or not? As the IFCC states in their recommendation, Part 1, that the term “reference” (as in “reference value”, “reference interval”, etc) should be preceded by a word qualifying the state of health (22). Terms such as “goal values” or “optimum values” may be considered (9). Strict interpretation of reference intervals should be avoided if the current individual is not free from diseases and/or medications and/or clinical examination tell something else about the individual. The best value to use as a reference is an earlier, but not too old, value of the person in question (9). If several previous values for the same analyte are available, it would be even better. Individual laboratory responses can often be interpreted on the basis of population-based reference intervals, when there is nothing else that they can be compared with (9, 23). The rapid development of personalized medicine may in the future point to the need for individual reference values (5). A reasonable conclusion when interpreting analytes in elderly individuals with disease or frailty is that individual evaluation based on the individual’s previous levels should be recommended (4, 9, 23).

Methodological considerations

Because the investigated NHRs in the NHR 2000 and NHR 2008 cohorts had different diseases and needed care all around the clock all, could be considered as frail, we paid a great deal of attention to collecting blood samples when they were in their habitual condition and were free from additional stresses, for example ongoing infection. The NHR 2008 cohort were closely monitored by the nursing staff who saw them daily and knew them well (3). Any sign of suspected infection received immediate attention and when infection was verified, blood samples were taken on later occasions, at least 2 weeks after treatment with antibiotics (e.g. in Paper III at baseline, and 6 and 12 months).

As a strength in the present thesis all laboratory analyses were performed in accredited laboratories, whose legitimized personnel perform their work according to rules and procedures as per the accreditation. This applies to instruments, reagents, internal and external controls, calibrators and consumables; furthermore, a large part of the work is that the laboratory should produce analytical values that are as accurate as possible, regardless of when and where the analysis is performed. However, the pre-analytical considerations, exact centrifugation times, sample storage, durability, and measurement uncertainty of a tested sample, also matter. Laboratory work also includes carefully controlled controls (CV, durability, time at room temperature before analysis, internal and external intervals, calibration to a traceable standard, accreditations and educations). Another strength is that the total database of the NORIP study has been used, not just their own calculations and proposed reference intervals.

In the present thesis, blood from the participating individuals was collected from different studies and the selection of anticoagulants differed. Thus, in the studies referred to as “NHR 2000” and “NHR 2008” (3), EDTA was used as anticoagulant, while in ELSA 85 (91) and NORIP studies (41), tubes without anticoagulant or addition of lithium heparin were used. In the present study, the EDTA anticoagulant limited the choice and number of investigated analytes, and five of the common laboratory analytes could be studied. The majority of the NHRs suffered from heart disease. Unfortunately, as the studies were restricted to the use of EDTA plasma, they therefore excluded analysis of analytes of interest in cardiac disorders. When using blood samples collected and frozen pre-analysis, the freezing period can affect the activity of the investigated analytes. Plasma from the study cohorts was analysed at different laboratories using measurement methods of different manufacturers, which could be a disadvantage. Nevertheless, the accreditation system includes traceability for the calibrators of the analytes, to the same references (33).

Ethical considerations

In the present thesis, data and blood samples from other studies (3, 41, 90, 91) were used. The participants (or the next of kin) gave consent to the use of their blood samples in these subsequent studies as well. Oral consent to collect blood samples was also obtained each time blood samples were taken. It was made clear that participation was voluntary and could be withdrawn at any time without giving reasons for withdrawal. From an ethical point of view, it must be considered positive to use blood samples that have been collected for other investigations and thus to avoid new blood sampling which can be experienced as disagreeable by the participants. It may be considered questionable to perform research in frail elderly individuals at all, but on the other hand, knowledge about laboratory levels in frail elderly people needs to increase and it would therefore be unethical to exclude them. It is hoped that the results might contribute to better knowledge and further improve the quality of life at the end of life. The studies complied with the Declaration of Helsinki (110).

Conclusions

In summary, the present research reveals that –

- Nursing home residents (NHRs) ≥ 80 years differ in levels of C3, C4, IgG, IgM, albumin, ALT, creatinine, sodium, phosphate and urea compared with values from both of the reference populations, blood donors and NORIP raw origin.
- Differences were found in mean values of albumin, ALT, AST, creatinine and γ -GT, when dividing individual's ≥ 80 years into “healthy”, “moderately healthy” and “frail”, based on ADL and MMSE scores.
- Elevated levels of CRP and IL-8 at baseline were related to shorter 8-year survival in ≥ 80 -year-old NHRs.
- Levels of Hb, RBCs and MCHC were lower after 1-year, but higher for mean corpuscular volume (MCV) and IL-1 β , compared to baseline or at 6 month follow-up, in NHRs ≥ 80 years.
- Levels of albumin, ALT, AST, creatinine and γ -GT were similar when comparing “healthy” and “moderately healthy” individuals in ≥ 80 -year-olds, with reference intervals proposed by NORIP study.
- In “frail” elderly individuals, regardless of classification model (ADL/MMSE or FI), levels of albumin, ALT, AST, creatinine and γ -GT differed most from reference intervals proposed by NORIP study and also from levels in “healthy” and “moderately healthy” ≥ 80 -year-olds.

Summary conclusion

Based on the present thesis it is clear that there is need for reference intervals that consider both age and health status in elderly individuals. A reasonable conclusion when interpreting levels of analytes in elderly individuals with disease or frailty is that individual evaluation based on the individual's previous levels is recommended.

Acknowledgements

Först vill jag tala om för alla som har stöttat mig under doktorandtiden och på ett eller annat sätt bidragit till arbetet med denna avhandling, hur oerhört tacksam jag är för det.

Till Ewa Grodzinsky, min huvudhandledare och mina bihandledare Märtha Sund-Levander, Anna Milberg och Jan Ernerudh. Med er entusiasm för forskning, den breda kunskapen som ni gärna delar med er av, tillsammans med tålamod som aldrig sinar, har ni varit ovärderliga för mig. Tack för allt vad ni lärt mig, både under givande och trevliga handledningar, men även oräkneliga tillfällen däremellan.

Tack till Pål Rustad, Først Medical Laboratory in Oslo, Norway, för att du delade med dig av bakgrundsdata för alla deltagare i NORIP studien, tillsammans med deras nivåer av laboratorieanalyser och gav tillåtelse att använda det i forskningssyfte i denna avhandling.

Rita Ylikevelä, som introducerade mig i statistikens underbara värld och då SPSS i synnerhet. Därefter har flera trevliga och duktiga personer med speciella kunskaper inom statistiken följt; Ann-Britt Wiréhn, Lasse Walter, Peter Garvin och Johan Lyth. Stort tack till er alla! Stort tack även till alla medarbetare på Enheten för forskningsstöd, som jag träffat genom året. Det har varit otroligt trevligt, inspirerande och lärorikt att få vistas i era lokaler tillsammans med er.

Tack till doktorander och seniora forskare vid Medicinska fakulteten vid Linköpings universitet och även andra universitet, för stöd och givande diskussioner.

Till mina fantastiska arbetskamrater på Laboratoriet och resten av Närsjukvården i Finspång, vill jag rikta ett stort tack. Det gäller inte minst Magnus Oweling, chef i Finspång, som hela tiden varit positiv och uppmuntrande och bidragit till att min forskning kunde bli möjlig.

Tack till Dzeneta Dernroth, Klinisk kemi, Linköping – för ditt trevliga bemötande och din vilja att hjälpa till, när hjälp har behövts. Tack till Hanna Gustafsson Bragde, Laboratoriemedicin, Ryhov, Jönköping – för introduktion i arbetet med Luminex och för trevligt omhändertagande när jag var och labbade hos er. Tack till Catarina Hellberg, Klinisk kemi, Eskilstuna – för trevligt bemötande och utmärkt hjälp när jag behövde få blodprover analyserad

Till alla mina släktingar och vänner som varit nyfikna, stöttat och kommit med glada tillrop, vill jag säga ett stort tack. Ett stort tack till mina föräldrar, systrar med familjer, som under hela mitt liv varit inspirerande och uppmuntrande, på olika vis.

Sist, min älskade familj: Tack till våra underbara barn, Linus och Johannes, för att ni är de ni är, helt fantastiska i allt vad ni är och gör. Tack min livskamrat, Göran, för ditt tålamod och uppmuntran, även om jag styrt med en massa saker de senaste åren som inte alltid varit varken lätta att förklara eller lätta att förstå. Du har alltid stöttat mig och det är jag innerligt tacksam för.

Avhandling har kunnat genomföras tack vare bidrag från Region Östergötland, Forskningsrådet i sydöstra Sverige (FORSS) samt Futurum – akademien för hälsa och vård Region Jönköpings län.

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