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# Reference values for 34 frequently used laboratory tests in 80-year-old men and women

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ABSTRACT

*Objectives:* Reference values are usually based on blood samples from healthy individuals in the age range 20–50 years. Most patients seeking health care are older than this reference population. Many reference intervals are age dependent and there is thus a need to have appropriate reference intervals also for elderly individuals.

*Methods:* We analyzed a group of frequently used laboratory tests in an 80-year-old population (n = 531, 266 females and 265 males). The 2.5th and 97.5th percentiles for these markers were calculated according to the International Federation of Clinical Chemistry guidelines on the statistical treatment of reference values.

*Results:* Reference values are reported for serum alanine transaminase (ALT), albumin, alkaline phosphatase, pancreatic amylase, apolipoprotein A1, apolipoprotein B, apolipoprotein B/apolipoprotein A1 ratio, aspartate aminotransferase (AST), AST/ALT ratio, bilirubin, calcium, calprotectin, cholesterol, HDL-cholesterol, creatinine kinase (CK), creatinine, creatinine estimated GFR, C-reactive protein, cystatin C, cystatin C estimated GFR, gamma-glutamyltransferase (GGT), iron, iron saturation, lactate dehydrogenase (LDH), magnesium, phosphate, transferrin, triglycerides, urate, urea, zinc, hemoglobin, platelet count and white blood cell count. The upper reference limit for creatinine and urea was significantly increased while the lower limit for iron and albumin was decreased in this elderly population in comparison with the population in the Nordic Reference Interval Project (NORIP).

*Conclusions:* Reference values calculated from the whole population and a subpopulation without cardiovascular disease showed strong concordance. Several of the reference interval limits were outside the 90% confidence interval of NORIP.



## 1. Introduction

Since the beginning of the 20th century there has been a dramatic increase in life expectancy [1]. Today life expectancy at birth exceeds 83 years in Japan and is more than 80 years in several other countries [1]. These are results of major improvements in health across the globe. Thus, we are getting older and at the same time healthier. Even if the elderly populations are healthier they seek health care more often than younger individuals.

Laboratory markers are an important part of clinical decision makings and the evaluation of test results is performed in relation to reference intervals provided by the laboratories. These reference intervals are often based on healthy individuals usually in the 20–50 year age range [2]. The reference interval is thus made for individuals that are younger than the average patient seeking health care. Changes in calibration of laboratory tests over time and the general improvement in health in the elderly populations also make old reference intervals less representative for the present elderly population. In Scandinavia many of the laboratories use reference intervals developed as part of the Nordic Reference Interval Project (NORIP) [3]. 102 laboratories in the Nordic countries collaborated to produce common reference intervals for 25 serum/plasma and 8 hematology methods. The individuals included in the reference intervals were adults below 70 years of age.

Many of the most widely used laboratory tests have been in clinical use for more than 50 years and are today well standardized with international calibrators. Internal and external quality assessment confirm that there is generally a good interlaboratory agreement between different manufacturers. It is thus in most cases possible to use common reference intervals. Several common reference values have also been established. A reference interval is based on results

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**Table 1**
Basic characteristics for the persons included in the reference material (n = 531). The results are presented as median and interquartile ranges (IQR). The interquartile ranges are shown within brackets. The prevalence of cardiovascular diseases and medication are presented as percentages.

| | Females, median (IQR) | Males, median (IQR) |
|---|---|---|
| Females/ males (number) | 266 | 265 |
| Without CVD (number) | 228 | 181 |
| Height (cm) | 160 (156–164) | 174 (170–179) |
| Weight (kg) | 68 (59–77) | 80 (72–87) |
| Waist circumference (cm) | 93 (86–101) | 98 (90–103) |
| Body mass index (BMI) (kg/m$^2$) | 26.2 (23.3–29.9) | 26.4 (24.0–28.8) |
| Waist/hip ratio | 0.91 (0.86–0.96) | 0.97 (0.93–1.01) |
| Diastolic blood pressure (mmHg) | 74 (68–78) | 74 (68–80) |
| Systolic blood pressure (mmHg) | 148 (136–162) | 142 (132–156) |
| Myocardial infarction | 4.9% | 14.7% |
| Stroke | 6.0% | 12.8% |
| Heart failure | 6.8% | 9.1% |
| Statin treatment | 27.4% | 32.5% |
| Warfarin | 8.6% | 17.7% |
| Diuretics | 27.1% | 17.0% |

from a "healthy population". Most elderly persons have today some kind of regular medication and many of them have signs of cardiovascular diseases (CVD). In a younger population such individuals are usually excluded when defining reference values but if we apply the same strict selection on an elderly population the remaining group will be very small and not very representative for elderly patients.

The aim of the present study was to find suitable reference values for an elderly population using a group of 80-year-old males and females and to study the effect of exclusion of individuals with known cardiovascular diseases on the reference intervals. We also wanted to compare reference intervals from NORIP with the corresponding values obtained in this elderly patient group.

## 2. Methods

### 2.1. Setting and participants

Seventy year old individuals living in Uppsala, Sweden, were originally included in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study [4]. Briefly, all subjects aged 70 years living in Uppsala, Sweden were eligible. The subjects were chosen from the community register and invited in a randomized order from the start of the study in April 2001 to the last included subject in June 2004. Of the 2025 subjects invited, 1016 subjects (507 men and 509 women) agreed to participate and were included in the study. All the participants gave their written informed consent and the Ethics committee of Uppsala University, Uppsala approved the study. The present study is from the reinvestigation of the cohort at the age of 80 years. Persons with a known diagnosis of diabetes or a fasting glucose value $\geq$7.0 mmol/L were excluded from this study. In total 531 individuals (266 females and 265 males) provided blood samples for this study. Basic characteristics of the study population are presented in Table 1.

### 2.2. Clinical and biochemical investigation

All blood samples were collected in the morning after an overnight fast. No medication or smoking was allowed after midnight. The whole blood samples were collected in $K_2$-EDTA tubes (Becton, Dickinson, Franklin Lakes, NJ, USA) and subsequently used for analysis of B-hemoglobin, B-white blood cell counts and B-platelet counts. The serum samples were collected in Vacutainer® tubes without additives (Becton, Dickinson). The serum samples were centrifugated for 20 min at room temperature and 2010 × *g*, frozen in multiple tubes to avoid repeated freezing and thawing and stored at −80 °C until analysis.

Alanine transaminase (ALT-8L92), albumin (7D54), alkaline phosphatase (7D55), pancreatic amylase (6K22), apolipoprotein A1 (9D92), apolipoprotein B (9D93), apolipoprotein B/apolipoprotein A1 ratio, aspartate aminotransferase (AST-8L91), AST/ALT ratio, bilirubin (6L45), calcium (3L79), calprotectin (Gentian, Moss, Norway), cholesterol (7D62), high-density lipoproteins (HDL)-cholesterol (3K33), creatinine (8L24, enzymatic method), creatinine kinase (CK-7D63), C-reactive protein (CRP-6K26), cystatin C (1101, Gentian), gamma-glutamyltransferase (GGT) (7D65), iron (6K95), iron saturation, lactate dehydrogenase (JDH-2P56), magnesium (3P68), phosphate (7D71), transferrin (1E04), triglycerides (7D74), urate (3P39), urea (7D75), zinc (17255, Sentinel Diagnostics, Milan, Italy), were analyzed on a BS380 instrument (Mindray, Shenzhen, China). The reagents were from Abbott Laboratories, Abbott Park, IL, US if not otherwise specified. Glomerular filtration rate (GFR) in mL/min/1.73m$^2$ was calculated from creatinine results using the CKD-EPI equation [5] and from cystatin C results by the CAPA equation [6].

Hemoglobin, platelet count and white blood cell count were analyzed with a CELL-DYN Sapphire Hematology System (Abbott Laboratories). Cardiovascular disease was defined as myocardial infarction, stroke and heart failure.

### 2.3. Statistical analysis

Calculations of reference intervals and 90% confidence intervals were performed by bootstrap estimation utilising RefVal 4.0 (Department of Clinical Chemistry, Rikshospitalet, N-0027 Oslo, Norway) [7,8]. The determination and evaluation of equality of the reference intervals were performed according to Clinical Laboratory Standards Institute guidelines EP28-A3C [9]. The Lahti et al. method described in EP28-A3C was used to test (0.9%–4.1%) if there were differences between reference intervals calculated with and without exclusion of individuals with CVD and differences from NORIP values [10].

### 2.4. Ethical issues

The study was approved by the ethics committee of the Faculty of Medicine, Uppsala University, and all participants gave informed consent prior to inclusion. The study was conducted according to the Declaration of Helsinki.

## 3. Results

### 3.1. Description of the study population

Basic characteristics of the study population and prevalence of cardiovascular diseases and medication are presented in Table 1. After the exclusion of individuals with diabetes the study population consisted of 266 females (228 without CVD) and 265 males (181 without CVD).

### 3.2. Reference intervals for males and females

The upper and lower limits for the reference intervals (2.5 and 97.5 percentiles and 90% confidence intervals) for males and females with and without CVD are presented in Table 2 together with the corresponding NORIP values. Limits that differ significantly from NORIP values according to Lahti et al. [10] are marked with † and differences between study populations with and without individuals with CVD are marked with £.