

SENIORLAB: a prospective observational study investigating laboratory parameters and their reference intervals in the elderly

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Abstract

Background: In clinical practice, laboratory results are often important for making diagnostic, therapeutic, and prognostic decisions. Interpreting individual results relies on accurate reference intervals and decision limits. Despite the considerable amount of resources in clinical medicine spent on elderly patients, accurate reference intervals for the elderly are rarely available. The SENIORLAB study set out to determine reference intervals in the elderly by investigating a large variety of laboratory parameters in clinical chemistry, hematology, and immunology.

Methods/design: The SENIORLAB study is an observational, prospective cohort study. Subjectively healthy residents of Switzerland aged 60 years and older were included for baseline examination (n = 1467), where anthropometric measurements were taken, medical history was reviewed, and a fasting blood sample was drawn under optimal preanalytical conditions. More than 110 laboratory parameters were measured, and a biobank was set up. The study participants are followed up every 3 to 5 years for quality of life, morbidity, and mortality. The primary aim is to evaluate different laboratory parameters at age-related reference intervals. The secondary aims of this study include the following: identify associations between different parameters, identify diagnostic characteristics to diagnose different circumstances, identify the prevalence of occult disease in subjectively healthy individuals, and identify the prognostic factors for the investigated outcomes, including mortality.

Discussion: To obtain better grounds to justify clinical decisions, specific reference intervals for laboratory parameters of the elderly are needed. Reference intervals are obtained from healthy individuals. A major obstacle when obtaining reference intervals in the elderly is the definition of health in seniors because individuals without any medical condition and any medication are rare in older adulthood. Reference intervals obtained from such individuals cannot be considered representative for seniors in a status of age-specific normal health. In addition to the established methods for determining reference intervals, this longitudinal study utilizes a unique approach, in that survival and long-term well-being are taken as indicators of health in seniors. This approach is expected to provide robust and representative reference intervals that are obtained from an adequate reference population and not a collective of highly selected individuals.

Trial registration: The present study was registered under International Standard Randomized Controlled Trial Number registry: ISRCTN53778569.

Abbreviations: CLSI = Clinical Laboratory Standards Institute, WHO = World Health Organization.

Keywords: laboratory parameters, morbidity, mortality, observational study, quality of life, reference intervals, senior, study protocol

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1. Background

In clinical medicine, decisions are often made based on patient history and examination results. Based on such types of information, prognosis and disease course can be estimated, and treatment decisions can be made.^[1] Within this context, laboratory medicine frequently provides important supportive information, on which decisions are based. In approximately 50% to 79% of hospitalized patients or outpatient cases from general internal medicine providers, laboratory measurements play a decisive role when planning the treatment course for the patient.^[2–4]

Laboratory test results are interpreted in the context of clinical information by comparison with reference intervals and longitudinal interpretation of sequential results.^[5–7] These reference intervals are most often obtained from investigations in healthy adult persons aged <60 years.^[8,9] Although elderly persons sometimes are regarded as elderly versions of adults, from a physiopathological viewpoint, seniors—analogously to children—have their distinct own biology. Despite this, reference intervals obtained from young and healthy persons are often used in elderly patients.^[10–12]

The importance of building reliable and robust reference intervals in laboratory parameters has recently gained increasing attention.^[13] In 2005, the International Federation of Clinical Chemistry and Laboratory Medicine founded a working group on reference ranges, and its publications have laid a foundation for the investigation of reference intervals.^[13–15] Although the majority of health care resources are spent on the elderly, reference ranges for the elderly are scarce. Thus far, with the exception of the very recently published data obtained within the framework of the Canadian Health Measures Survey,^[16–18] few studies have aimed to define normal ranges for the most common analyses, which included <40 parameters in each study.^[19–26] Some of this scientific work has been published >20 years ago and has been conducted in relatively small cohorts. As a major drawback of these studies, the employed laboratory methods cannot be traced to reference methods or are not in use anymore. Furthermore, all of these investigations are cross-sectional in nature. Accordingly, the usefulness of the reference intervals of the reported parameters can be considered to be limited.

Wrong interpretation of laboratory test results due to the use of unsuitable reference ranges leads to false-negative and false-positive results. False results are a major source of harm in a patient. Time-consuming and worrying test procedures are too often performed in medicine to substantiate (false) positive findings. These procedures are not only burdensome for the patient and his or her family caregivers but also a major cause of unnecessarily expenses. At the end of life, the aim is to limit medical procedures to the minimum. Unnecessary procedures in this phase of life can be considered unethical because they put an unnecessary burden on the shoulders of a patient whose life should be made as comfortable as possible. As long as clinical medicine uses inappropriate reference intervals for the elderly, unnecessary procedures may be commonly ordered. More appropriate reference intervals are expected to reduce the frequency of potentially humiliating medical procedures during the last phase of life.^[1]

To obtain better grounds on which to base clinical decisions, specific reference intervals for frequently used laboratory parameters of the elderly are urgently needed.^[10–12] The present study protocol presents the SENIORLAB study as an investigation with an extensive examination of study participants at baseline and, as a further unique feature of this study, a longitudinal follow-up.

The study setting allows for work on several aims and investigation of a multitude of research questions. The primary aim is to establish robust reference intervals for >110 different parameters in subjectively healthy elderly persons who demonstrated survival at the follow-up examinations. This additional methodological strength of the SENIORLAB study is unprecedented and has the potential of modifying the theory of constructing reference intervals within the senior citizen community. The secondary aims of the SENIORLAB study are as follows: to test the diagnostic characteristics of certain laboratory parameters (e.g., red blood cell characteristics) and to characterize biochemically defined disorders such as vitamin B₁₂ deficiency, which can also occur in a subjectively healthy elderly cohort (this approach may help identify novel markers for more efficient identification of common disorders among the elderly population); to investigate associations of different biomarkers with other biomarkers (this approach helps to better understand the pathophysiology and behavior of the different laboratory parameters in seniors and may ultimately lead to a better interpretation of laboratory results, preventing false-negative and

false-positive diagnosis in elderly patients with the subsequent consequences); to investigate the prevalence of occult and early disease in subjectively healthy elderly persons (e.g., type 2 diabetes mellitus, thyroid disease, vitamin deficiency, renal disease) (some disorders are exclusively diagnosed by laboratory parameters; this approach helps estimate the magnitude of relevant underdiagnosed diseases in the elderly and may help shape policies regarding better and earlier detection for earlier intervention); and to investigate risk factors for the loss of subjective well-being, mortality, hospitalization, and impaired autonomy among the elderly. Identifying these factors may help identify elderly individuals who may appear healthy but require closer medical attention and may benefit from medical interventions.

2. Methods/design

2.1. Study design

Reporting the design of this study strictly follows the STROBE statement, except for the guidelines in results and discussion sections because this report is a study protocol.^[27] The research project is designed as a prospective, single-center, cohort study with an extensive baseline examination and a periodic longitudinal follow-up. The current article provides a comprehensive synopsis for the main design of the SENIORLAB study, including a report on participant recruitment, baseline examinations, and follow-up.

2.2. Setting

The study was initiated by a medical laboratory and aimed to recruit study participants in the community. The location and organization of the study center is associated with the laboratory, which is located in Berne in the midlands of Switzerland. For the baseline examination, study participants were recruited from May 2009 to December 2011. Follow-up examinations are planned every 3 to 5 years. The first follow-up was done from December 2013 to December 2014. The second follow-up investigation is scheduled for October 2017 to October 2018. The study is registered in the International Standard Randomized Controlled Trial Number registry (ISRCTN53778569), where all items of the World Health Organization (WHO) Trial Registration Data Set have been specified.

2.3. Ethics, consent, and permissions

The study was approved by the Ethics Committee Bern (Kantonale Ethikkommission Bern; ref 166/08) on January 5, 2009, and an amendment regarding the prospective study was approved on July 29, 2013. Study participants provided written informed consent before entering the study.

2.4. Participants

Consecutive, subjectively healthy elderly volunteers aged 60 years and older were recruited. The study participants were contacted through newspaper advertisements, various clubs, and at associations that had high proportions of healthy elderly members (e.g., alpine clubs and sports clubs). In addition, participants were recruited through personal contacts of the collaborators of the study organization. Inclusion criteria were as follows: age 60 or older, residence in Switzerland, the subjective perception of being healthy, and being in a fasting state at the

baseline examination. Exclusion criteria were as follows: known diabetes mellitus, known thyroid disease, current glucocorticoid use, active neoplastic disease during the past 5 years, consumption of >5 pharmacologically active substances (polypharmacy), and hospitalization during the past 4 weeks. Study participants and their primary care physicians, as requested, were given the results of selected values that are widely screened in asymptomatic healthy individuals, such as fasting glucose, total cholesterol, creatinine, hemoglobin, red blood cells, white blood cells, and thrombocytes. For the follow-up examinations, study participants are contacted by letter and telephone. In the case of a participant's lack of response, official communal authorities, relatives, and/or neighbors are contacted to obtain the study participant's information.

2.5. Data collection and measurements

Each participant's personal history and anthropometric measurements, such as height and weight, were obtained. Histories of participants were obtained using questions including whether a participant considered himself or herself as healthy and believed to have an intact cognitive state. Furthermore, a personal history of diseases and surgical procedures, as well as intake of drugs and supplements, was recorded. Blood pressure was measured in a standardized manner in a seated position after a 10-minute rest. Venous blood was drawn into S-Monovette (Sarstedt, Sevelen, Switzerland) after an overnight fasting period. The laboratory samples were processed (i.e., centrifuged, aliquoted, and analyzed or frozen at -80°C) immediately after the blood was drawn to allow for standardized preanalytics. In the follow-up surveys, information on subjective well-being, mortality, reason of death, hospitalization, and impaired autonomy is recorded.

The investigators will have access to the final dataset. For qualified analysis plans, they will provide data access to qualified scientists. The data monitoring committee consists of the 3 study investigators and ascertains integrity as well as correctness of data and analyzed and handled adverse events and other unintended effects. This was done after the baseline examination and will be continued after each follow-up period. Follow-up will be performed until funds are available. Confidentiality of data is protected before, during, and after the trial.

Laboratory parameters were measured on up-to-date diagnostic platforms from different manufacturers (e.g., Roche Diagnostics, Abbott Diagnostics, Siemens, Beckmann Coulter, Sysmex) and also by employing, for example, open immunoassay platforms or high-performance liquid chromatography-tandem mass spectrometry instruments. The laboratory analyses were performed according to the requirements of the Swiss commission for quality assurance in the medical laboratory. For internal quality control, commercially available control materials were used when available. The investigated parameters, together with manufacturer and imprecision, are provided in Table 1. If available, aliquots of each sample were stored at -80°C . This step allows for the analysis of additional parameters of interest. The freezers used to store the samples have been continuously monitored for an adequate temperature.

2.6. Sample size

The Clinical Laboratory Standards Institute (CLSI) released a relevant guideline (C28-A3c) for the evaluation of reference intervals.^[15] There are several possibilities to evaluate reference intervals. The most commonly stated minimal number of

included subjects is 120 per stratum. This number allows for determination of the 90% confidence interval of the upper and lower reference limits. However, the so-called robust method also offers the possibility to evaluate reference intervals with fewer individuals. We anticipated age and gender stratification. For age stratification, we collapsed the participants to 5-year age cohorts (e.g., age 60–64, 65–69, 70–74). We aimed to include at least 120 participants in the 5-year age cohorts. The recruitment for an age stratum was stopped if >200 participants were already included in the respective stratum.

2.7. Analysis plan

An overview on participant enrollment, data acquisition, and data analysis is given in the flow diagram (see Fig. 1). Participants can be excluded at different stages of the study: before baseline examination or after the baseline visit. Clinicopathological measurements were conducted immediately after drawing the blood samples to provide optimal preanalytical conditions. Due to logistical reasons, it sometimes only became apparent that an exclusion criterion applied after clinicopathological measurements were conducted (e.g., in the case of consumption of a combination drug or in the case of the consumption of an antidiabetic medication in the absence of information that a participant suffers from diabetes, despite explicitly being asked about this condition). Together, 1467 individuals presented at the baseline visit. For the determination of reference intervals, participants lost to follow-up will not be included into the statistical analysis.

To evaluate reference intervals (primary aim) according to the CLSI C28-A3c guideline, laboratory parameters will be analyzed for partitioning factors (e.g., age, gender) by correlation in the case of continuous variables and by a statistical comparison of the means and medians in the case of binary variables. Outliers will be eliminated according to the method of Dixon and Reed or alternative methods.^[15] After an analysis of the normal distributions of the laboratory results, parametric or nonparametric reference ranges will be calculated. Should a stratum have fewer than 120 subjects, then the robust method will be applied. Judgment on whether the included number of study participants in a respective stratum is sufficient will be made by the method of Boyd and coworkers.^[15] Furthermore, age-related continuous reference intervals are calculated.^[28–30] The computer programs MedCalc (Mariakerke, Belgium) and StatisPro (CLSI, Wayne, PA) will be used for these calculations and analyses.^[15]

For statistical analysis of the secondary aims, the following methods will be used: diagnostic characteristics will be evaluated by Receiver operating characteristic curve analysis, and sensitivity, specificity, predictive values, and likelihood ratios will be calculated (by looking at optimum decision concentrations and concentrations with high sensitivity and high specificity, this approach may be able to evaluate decision limits that identify persons with probable or improbable disease and describe a gray zone of diagnostic uncertainty)^[31]; associations between different variables will be assessed by correlation and by univariate and multivariate linear regression analyses (the latter will represent a means to control for covariates, confounders, or potential bias; goodness of model fit, regression coefficients, and partial correlation coefficients will be evaluated in these models); descriptive statistics will be used to evaluate the frequency of occult disease; logistic regression analysis and longitudinal analysis will be employed to evaluate risks for the occurrence of outcomes.

Table 1**Employed laboratory methods with their imprecision at different concentration levels.**

Analyte	Manufacturer	Method	Method	Unit	Mean 1	CV 1, %	Mean 2	CV 2, %	Mean 3	CV 3, %
ALT	Roche Diagnostics	INTEGRA800	IFCC method, without pyridoxine-5'-phosphate	U/L	21.4	3.2	76.2	2.4	169.3	2.60
Albumin	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	g/L	25.2	2.3	33.9	2.0	42.054	2.00
Aldosterone	Siemens	Packard RIA StAR	Radioimmunoassay	pmol/L	146	7.1	487	3.8	1486	3.00
Alkaline phosphatase	Roche Diagnostics	INTEGRA800	Colorimetric assay IFCC standardized	U/L	31.1	5.5	147.5	1.5	282.3	2.20
α_1 -Antitrypsin	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.76	4.6	1.35	5.7	1.85	5.80
α_1 -Acid glycoprotein	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.561	5.8	0.704	5.7	1.05	5.70
Ammonia	Roche Diagnostics	INTEGRA800	Enzymatic assay with glutamate dehydrogenase	μ mol/L	69.9	6.8	250.5	2.8		
Amylase	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay	U/L	43.6	2.1	135.3	2.0	278.7	2.10
Antinuclear antibody	Euroimmun	Sprinter	Indirect immunofluorescence microscopy	Titer	Negative	n.a.	Positive	n.a.		
Apolipoprotein A	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	g/L	0.935	4.3	1.92	4.4	2.7	3.40
Apolipoprotein B	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	g/L	0.399	6.5	0.904	5.4	1.32	4.90
AST	Roche Diagnostics	INTEGRA800	Method according to IFCC, without pyridoxine-5'-phosphate	U/L	41.1	2.6	105.3	2.7	249.2	2.50
Basophils	Sysmex	XE-5000	Flow cytometry	%	63	2.2	67.2	1.1	71.8	0.90
β_2 microglobulin	Siemens	Immulite 2000	Luminescent immunoassay	mg/L	29	9.5	77	8.2	323	10.30
BNP	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	pg/mL	91.9	8.3	484	6.8	3368	2.90
Complement C3	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.82	4.2	1.76	4.9	2.61	4.00
Complement C4	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.161	4.8	0.38	3.4	0.551	6.00
Calcium	Roche Diagnostics	INTEGRA800	Schwarzenbach method with α -cresolphthalein complexone (colorimetric?)	mmol/L	1.40	1.7	2.54	1.6	3.24	1.80
Coeruloplasmin	Dade Behring	BN ProSpec	Nephelometry	g/L	0.189	5.4	0.36	7.2	0.496	5.60
Chloride	Roche Diagnostics	INTEGRA800	Indirect ion selective electrode	mmol/L	73.6	1.7	98.4	1.5	120.2	1.60
Cholesterol	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay (CHOD/PAP) with cholesterinesterase	mmol/L	2.584	2.5	4.747	1.9	6.802	2.10
Cholinesterase	Roche Diagnostics	INTEGRA800	Colorimetric assay with s-butrylthiocholine iodide	U/L	5355	2.6				
Cortisol	Siemens	Immulite 2000	Luminescent immunoassay	nmol/L	135.6	7.3	566.5	7.4	1035.0	6.30
C-reactive protein	Roche Diagnostics	INTEGRA800	PETIA	mg/L	6.178	3.6	27.056	4.4	44.247	3.20
Creatine kinase	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay IFCC	U/L	78.0	4.8	257.6	1.2	597.5	1.30
Creatinine	Roche Diagnostics	INTEGRA800	Kinetic colorimetric assay (Jaffe method)	μ mol/L	49.5	4.8	151.6	3.5	518.1	2.90
C-terminal telopeptide	Roche Diagnostics	E 170	Electroch-0 emiluminescence immunoassay	ng/mL	24.9	1.5	79	1.6		
Cystatin C	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	mg/L	0.352	6.7	0.509	4.2	0.636	4.10
Direct bilirubin	Roche Diagnostics	INTEGRA800	Diazo colorimetric method	μ mol/L	6.0	8.6	42.5	2.1	97.2	1.50
Eosinophils	Sysmex	XE-5000	Flow cytometry	%	9.3	11.4	10.5	9.4	11.9	6.50
E2	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	pmol/L	109	9.8	845	2.6	1920	2.40
ENA: anticentromere B antibodies	BioRad	BioPlex 2200	Bead array	Index	2.65	9.8				
ENA: antichromatin antibodies	BioRad	BioPlex 2200	Bead array	Index	2.66	10.1				
ENA: anti-dsDNA antibodies	BioRad	BioPlex 2200	Bead array	IU/mL	27.85	11.5				
ENA: anti-Jo1 antibodies	BioRad	BioPlex 2200	Bead array	Index	2.62	10.1				
ENA: antiribosomal P protein antibodies	BioRad	BioPlex 2200	Bead array	Index	2.35	9.4				
ENA: anti-RNP-68 antibodies	BioRad	BioPlex 2200	Bead array	Index	2.47	7.8				
ENA: anti-RNP-A antibodies	BioRad	BioPlex 2200	Bead array	Index	2.34	7.6				
ENA: anti-Scl70 antibodies	BioRad	BioPlex 2200	Bead array	Index	2.42	7.8				

Analyte	Manufacturer	Method	Method	Unit	Mean 1	CV 1, %	Mean 2	CV 2, %	Mean 3	CV 3, %
ENA: anti-Sm antibodies	BioRad	BioPlex 2200	Bead array	Index	2.17	11.3				
ENA: anti-Sm/RNP antibodies	BioRad	BioPlex 2200	Bead array	Index	2.99	11				
ENA: anti-SSA 52 antibodies	BioRad	BioPlex 2200	Bead array	Index	2.44	11.9				
ENA: anti-SSA 60 antibodies	BioRad	BioPlex 2200	Bead array	Index	2.58	9.4				
ENA: anti-SSB antibodies	BioRad	BioPlex 2200	Bead array	Index	2.66	8				
Ferritin	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	ng/mL	26.8	5.8	196.5	4.7	460	5.10
Folic acid	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	nmol/L	6.6	10.4	18.9	8.7	25.1	8.10
Folic acid in Ec Free T3	Beckman Coulter Abbott	UniCel Dxl 800 Architect i4000	Luminescent immunoassay Chemiluminescent microparticle immunoassay	nmol/L pmol/L	3.76	8.5	9.64	5.4		
Free T4	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	pmol/L	13.7	5.7	31.4	6.3		
Fructosamine	Roche Diagnostics	INTEGRA800	Colorimetric assay by reaction with nitro blue tetrazolium	μmol/L	253.2	3.1	499.5	2.2		
γ-Glutamyl transferase	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay standardized by Szasz	U/L	27.9	4.6	77.2	3.6	127.6	3.60
Glucose	Roche Diagnostics	INTEGRA800	Enzymatic reference method with hexokinase	mmol/L	3.301	2.3	6.477	1.7	19.655	1.50
Haptoglobin	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.683	7.7	1.562	4.0	2.43	5.60
HbA1c	Bio-Rad	D10	High-performance liquid chromatography	%	5.83	3.9	9.952	2.5		
Hematocrit	Sysmex	XE-5000	Impedance measurement	%	17.6	0.7	35.3	0.4	48.4	0.60
Hemoglobin	Sysmex	XE-5000	Photometry	g/L	58	0.7	120	0.5	170	0.40
Holotranscobalamin	Abbott	AxSYM	Microparticle enzyme immunoassay	pmol/L	18.9	6.4	46.1	5.8		
Homocysteine	Siemens	Immulite 2000	Luminescent immunoassay	μmol/L	12.9	9.3	23.7	8.0		
IgA	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	1.153	3.8	2.599	4.5	3.966	3.20
IgE	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	58	5.5	118	5.4	161	5.20
IgG	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	9.64	4.1	16.518	8.2	23.302	5.80
IgG1 subclass	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	3.9	3.7	6.1	4.8	7.7	3.20
IgG2 subclass	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	2	5.5	3.2	5.9	3.5	4.00
IgG3 subclass	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.25	4.8	0.4	6.2	0.55	4.40
IgG4 subclass	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.41	4.2	0.66	3.8	0.81	2.30
IgM	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.673	3.7	1.64	5.0	2.519	4.20
Insulin	Roche	Cobas 6000	Electrochemiluminescence immunoassay	mIU/L	21	2.08	72.6	2.63		
Iron	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay (guanidine/ferrozine method)	μmol/L	12.6	3.2	27.1	1.7	41.6	1.60
LDH	Roche Diagnostics	INTEGRA800	Optimized standardized method according to DGKC	U/L	225.5	2.5	317.8	2.1	709.6	1.80
Leukocytes (WBC)	Sysmex	XE-5000	Flow cytometry	10 ³ /μL	3.01	2.9	6.58	2.2	17.5	0.90
Lipase	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay (with 1,2-O-dilauryl-rac-glycerol-3-glutaric acid resorufin ester as substrate)	U/L	154.5	2.7	49.4	3.0	33.2	3.90
Lipoprotein a	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	nmol/L	304	6.6				
HDL	Roche Diagnostics	INTEGRA800	Homogeneous enzymatic colorimetric assay	mmol/L	0.777	2.7	1.191	2.4	1.522	2.90
LDL	Roche Diagnostics	INTEGRA800	Homogeneous enzymatic colorimetric assay	mmol/L	1.352	2.4	2.567	2.2	3.895	2.00
Lymphocytes	Sysmex	XE-5000	Flow cytometry	%	35.3	3.9	30.8	1.4	25.9	2.10
Magnesium	Roche Diagnostics	INTEGRA800	Colorimetric assay with chlorophosphonazo III	mmol/L	0.462	2.0	1.039	1.4	1.583	1.10
MCH	Sysmex	XE-5000	Photometry	pg	74.2	0.4	78.9	0.3	90.5	0.30
MCHC	Sysmex	XE-5000	Photometry	g/L	330	0.7	339	0.6	350	0.80
MCV	Sysmex	XE-5000	Impedance measurement	fL	74.2	0.4	78.9	0.3	90.5	0.30
MMA	LC-MS/MS	SCIEX API 4000 LC-157 MS/MS	LC-MS/MS	nmol/L	380	1.4	661	1.8		

Analyte	Manufacturer	Method	Method	Unit	Mean 1	CV 1, %	Mean 2	CV 2, %	Mean 3	CV 3, %
Monocytes	Sysmex	XE-5000	Flow cytometry	%	9.5	7.9	9.6	6.4	9.5	7.90
Neutrophils	Sysmex	XE-5000	Flow cytometry	%	45.9	3.1	49.1	3.0	51.6	2.40
Ostase	Beckman Coulter	UniCel Dxl 800	Luminescent immunoassay	μg/L	10	7.7	44.2	7.5		
Pancreatic amylase	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay	U/L	40.8	2.4	108.5	2.5		
Parathyroid hormone	Siemens	Immulite 2000	Luminescent immunoassay	pmol/L	5.9	6.1	36.3	6.6		
Phosphate	Roche Diagnostics	INTEGRA800	Endpoint method	mmol/L	0.662	2.4	1.409	1.5	2.383	1.40
Platelets	Sysmex	XE-5000	Flow cytometry	10 ³ /μL	55	1.8	213	1.7	510	0.90
Potassium	Roche Diagnostics	INTEGRA800	Indirect ion selective electrode	mmol/L	2.271	1.8	3.68	1.1	7.32	1.60
Prostate-specific antigen	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	μg/L	0.344	5.0	2.308	5.2	20.23	5.30
Red blood cell count (erythrocytes)	Sysmex	XE-5000	Impedance measurement	10 ⁶ /μL	2.38	0.4	4.47	0.5	5.35	0.50
Reticulocyte hemoglobin	Sysmex	XE-5000	Impedance measurement	pg	25	1.5	25.8	1.6	27.8	0.80
Reticulocytes	Sysmex	XE-5000	Flow cytometry	%	6.78	1.8	2.54	2.8	0.99	4.90
Retinol-binding protein	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	g/L	0.03	7.4	0.05	8.1	0.07	7.10
Rheumatoid factor	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	U/mL	29	2.9	47.652	3.1	52.467	3.10
Sensitive C-reactive protein	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	mg/L	1.716	8.7	13.467	5.3		
SHBG	Siemens	Immulite 2000	Luminescent immunoassay	nmol/L	3.98	5.8	75.4	6.3		
Sodium	Roche Diagnostics	INTEGRA800	Indirect ion selective electrode	mmol/L	107	1.7	138	1.2	159	1.60
Testosterone	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	nmol/L	3.6	9.7	15.8	4.4	35.9	3.20
Total bilirubin	Roche Diagnostics	INTEGRA800	Colorimetric assay	μmol/L	3.6	6.3	22.3	2.8	44.5	2.70
Total protein	Roche Diagnostics	INTEGRA800	Colorimetric assay	g/L	37.7	1.9	50.6	1.5	63.7	1.20
Transferrin	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	g/L	1.611	1.9	2.753	1.9	3.862	1.60
Transferrin receptor, soluble	Siemens Healthcare Diagnostics	BN ProSpec	Nephelometry	mg/L	0.75	1.5	1.28	3.6	1.51	4.00
Transthyretin (prealbumin)	Roche Diagnostics	INTEGRA800	Turbidimetric inhibition immunoassay	g/L	0.145	3.5	0.241	2.9	0.335	3.10
Triglyceride	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazon	mmol/L	0.933	2.7	1.451	2.0	2.127	1.90
TSH	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	mU/L	0.72	4.7	5.26	4.2	26.4	3.90
Urea	Roche Diagnostics	INTEGRA800	Kinetic assay (with urease and glutamate dehydrogenase)	mmol/L	5.1	3.2	13.4	2.7	25.5	2.40
Uric acid	Roche Diagnostics	INTEGRA800	Enzymatic colorimetric assay (with uricase and 4-aminoantipyrine)	μmol/L	202.5	2.4	358.3	2.2	541.9	2.10
Uromodulin	BioVendor	Dynex	Enzyme linked immunoassay	ng/mL	3	4	10.1	6.7		
Vitamin B ₁₂	Abbott	Architect i4000	Chemiluminescent microparticle immunoassay	pmol/L	162.2	6.6	332.7	6.7	415.4	6.00
25-OH vitamin D	Recipe	High-performance liquid chromatography	High-performance liquid chromatography	μg/L	25.6	4.3	85.7	4.1		

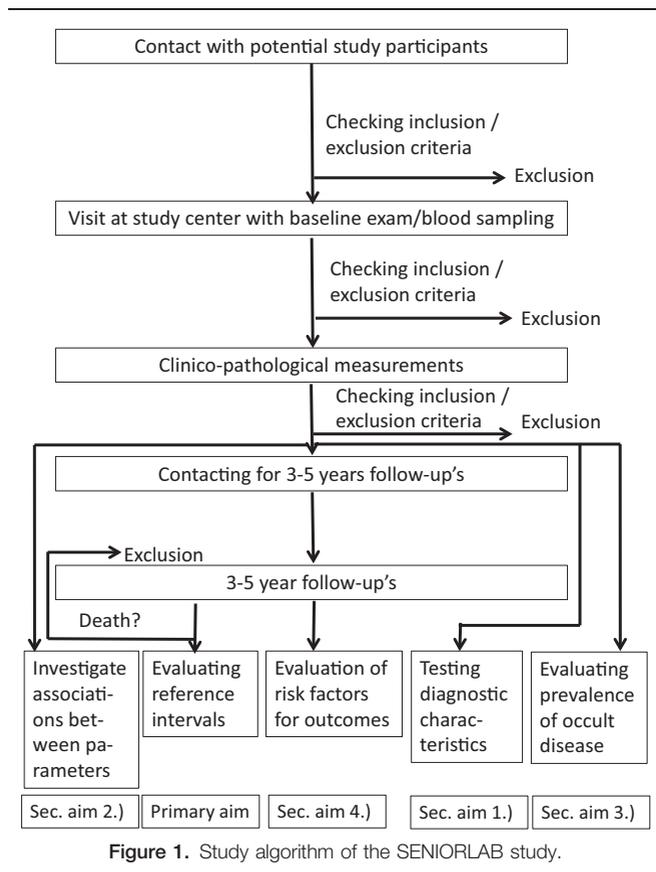
ALT = alanine transaminase, AST = aspartate transaminase, BNP = brain natriuretic peptide, C3 = complement C3, C4 = complement C4, CHOD-PAP = cholesterol oxidase/peroxidase aminophenazon, CV = coefficient of variation, DGKC = Deutsche Gesellschaft für Klinische Chemie, dsDNA = double stranded deoxyribonucleic acid, E2 = estradiol, Ec = erythrocyte, ENA = extractable nuclear antigen, GPO-PAP = Glycerol phosphate oxidase/peroxidase aminophenazon, HbA1c = glycated hemoglobin, HDL = high-density lipoprotein, IFCC = International Federation of Clinical Chemistry, Ig = immunoglobulin, LC-MS/MS = liquid chromatography mass spectrometry, LDH = lactate dehydrogenase, LDL = low-density lipoprotein, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, MMA = methylmalonic acid, n.a. = not applicable, PETIA = particle-enhanced turbidimetric immunoassay, SHBG = sex hormone-binding globulin, TSH = thyroid-stimulating hormone, WBC = white blood cell.

For all other analyses, statistical comparisons will be performed by *t* test/Mann–Whitney *U* test, χ^2 or Fisher exact test; tests for trend; or analysis of variance/Kruskal–Wallis test, as indicated by the specific statistical problem. The computer programs MedCalc (Mariakerke) and SPSS (IBM, Zurich, Switzerland) will be used for the analyses of secondary aims. Study results will be disseminated via publications. Authorship will be attributed according to the criteria issued by the International Committee of Medical Journal Editors. Data will be made accessible to other researches after data sharing arrangements could be met.

3. Discussion

To our knowledge, the SENIORLAB study represents the largest and most comprehensive study aiming at establishing reference

intervals in the elderly thus far. Typically, reference interval studies are cross-sectional in nature and are conducted in healthy cohorts. According to the WHO, health is a status of subjectively perceived well-being: “health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.”^[32,33] Because seniors frequently suffer from ≥ 1 diseases without any effect on the subjective perception of health status (e.g., arterial hypertension), the definition of health represents a crucial and problematic point in establishing reference intervals in this age group.^[34] Normal senior reference subjects without any disease, medication, and supplementation are too rare and cannot be regarded representative for the group of “healthy seniors.”^[35] Therefore, novel approaches for selecting reference subjects are warranted for establishing reference intervals in seniors. In addition to the subjective perception of health, the SENIORLAB study, with its longitu-



dinal design, adds objectifying characteristics such as survival and long-term well-being.

The secondary aims of the SENIORLAB study will be able to clarify the physiology of different parameters, as it has already been illustrated by describing the importance of serum uromodulin, an analyte that is increasingly recognized for its importance in understanding the development of renal and cardiovascular disease and kidney function in healthy persons.^[36] Furthermore, parameters can be identified for the screening of commonly encountered disorders among the elderly population, such as using hematological indices to screen for common nutritional deficiencies of different vitamins and iron. Such an approach may help to increase the effective use of resources in the clinical laboratory. Associations between different parameters will help to better understand the pathophysiology of different parameters and allow for better interpretation.^[37,38] Finally, the investigation of the prevalence of occult disease in the elderly and the identification of longitudinal risks, such as mortality, is of great importance from a public health perspective. This approach is likely to indicate possibilities to antagonize adverse outcomes for individuals and the public. Together, the SENIORLAB study can provide important evidence in the following ways: for the principle and practice of using laboratory medicine resources, for identification of better index and screening tests, for better understanding of physiological behavior of laboratory parameters, for identifying underdiagnosed and undervalued diseases in the elderly, and for identifying important risk factors in the elderly.

The SENIORLAB study possesses strengths and limitations. Regarding the primary aim of reference interval determination,

internal validity is hampered by the fact that biological variation was not considered because, in most individuals, only 1 blood sample was obtained per participant and only a minority of participants provided 2 blood samples.^[39] As a consequence, the study cannot assess a preference of population-based reference intervals over intraindividual reference changing values.^[7] External validity is impaired by the fact that the investigated cohort consists entirely of participants with Caucasian descent. As a consequence, the evaluated reference intervals might not be extrapolated to persons with other racial backgrounds. Furthermore, the term of a subjectively healthy well-being is debatable and might also impair extrapolation to other groups. We intentionally chose a nonrestrictive definition of health, which in our view is closer to the majority of elderly persons. Absence of disease or medication in the elderly is relatively rare. For example, normal blood pressure is encountered in only 7% of persons aged ≥ 80 years.^[40] Furthermore, medication use in the elderly occurs very frequently, and surveys have shown that up to 80% in the age group of >65 years reported having ingested at least 1 drug during the past week.^[41]

The fact that a longitudinal follow-up has been undertaken differentiates the SENIORLAB study from all other studies investigating reference intervals in the elderly. In this age group, a life expectancy >3 years can be considered as a long-term life expectancy.^[42,43] Based on the difficulties in performing a clear-cut objective differentiation of health from disease in the elderly, we are convinced that long-term life expectancy in addition to perception of subjective health is a robust and suitable additive criterion to select individuals for the evaluation of reference intervals. This approach is well supported by the findings from the Cardiovascular Health Study, which demonstrated that elderly study participants >65 years who were in the lowest hemoglobin quintile had an increased risk for death in a fully adjusted model despite not fulfilling WHO but also other criteria for anemia (i.e., hemoglobin concentration below the lower limit of the hemoglobin reference interval).^[44-46] This study clearly suggests that mortality should be considered when defining reference ranges in the elderly.

We expect the SENIORLAB study to provide insights on the influence of age on the behavior of laboratory parameters. Accordingly, we will be able to define accurate reference intervals and decision limits for a multitude of laboratory parameters, which constitute a great majority of clinically ordered laboratory test in senior citizens. From a methodological perspective, we will be able to include longitudinal follow-up as a relevant criterion to select appropriate individuals for the evaluation of reference intervals. This follow-up will be an important step in the theory and practice of defining reference intervals in the elderly.

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References

- [1] Gross R, Löffler M. Prinzipien der Medizin- Eine Übersicht ihrer Grundlagen und Methoden. Berlin:Springer; 1997.

- [2] Gross R, Fischer R. Diagnosen am Beispiel einer Medizinischen Klinik. *Diagnostik* 1980;13:113–6.
- [3] Beeler I, Szucs T, Gutzwiller F. Is the general practice laboratory medically and economically valuable? *Praxis* 2001;90:887–96.
- [4] Risch L. Medizinische Validation. *Pipette-Swiss Laboratory Medicine* 2011;8:11.
- [5] Horn PS, Pesce AJ. Reference intervals: an update. *Clin Chim Acta* 2003;334:5–23.
- [6] Risch L, Gassner M, Warzinek T. Labormedizin- ein Fach an der Schnittstelle von Technologie und Medizin. *Fortschritt durch Bildung, Wissenschaft und Zusammenarbeit*. Sargans:Sarganserländer Verlag; 2013. 185–90.
- [7] Pineda-Tenor D, Laserna-Mendieta EJ, Timon-Zapata J, et al. Biological variation and reference change values of common clinical chemistry and haematologic laboratory analytes in the elderly population. *Clin Chem Lab Med* 2013;51:851–62.
- [8] Plebani M, Lackner K. Reference values: still a critical issue in laboratory medicine. *Clin Chem Lab Med* 2010;48:577–8.
- [9] Siest G. Biological reference values. *In Vitro Veritas*. 2001;2:4. Available at: <http://www.accl.cat/continguts/ivv025.pdf> [accessed 23.7.2014].
- [10] Sikaris KA. Physiology and its importance for reference intervals. *Clin Biochem Rev* 2014;35:3–14.
- [11] Kelso T. Laboratory values in the elderly. Are they different? *Emerg Med Clin North Am* 1990;8:241–54.
- [12] Cavalieri TA, Chopra A, Bryman PN. When outside the norm is normal: interpreting lab data in the aged. *Geriatrics* 1992;47:66–70.
- [13] Siest G, Henny J, Grasbeck R, et al. The theory of reference values: an unfinished symphony. *Clin Chem Lab Med* 2013;51:47–64.
- [14] Ichihara K, Boyd JC. IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) An appraisal of statistical procedures used in derivation of reference intervals. *Clin Chem Lab Med* 2010;48:1537–51.
- [15] Horowitz GL, Altaie S, Boyd JC, et al. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline*. 3rd ed, vol 28 Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
- [16] Adeli K, Higgins V, Nieuwesteeg M, et al. Complex reference values for endocrine and special chemistry biomarkers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian Health Measures Survey. *Clin Chem* 2015;61:1063–74.
- [17] 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. *Clin Chem* 2015;61:1049–62.
- [18] Adeli K, Raizman JE, Chen Y, et al. Complex biological profile of hematologic markers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian Health Measures Survey. *Clin Chem* 2015;61:1075–86.
- [19] Carlsson L, Lind L, Larsson A. Reference values for 27 clinical chemistry tests in 70-year-old males and females. *Gerontology* 2010;56:259–65.
- [20] Garry PJ, Hunt WC, Vanderjagt DJ, et al. Clinical chemistry reference intervals for healthy elderly subjects. *Am J Clin Nutr* 1989;50(5 suppl):1219–30. [discussion 1231–1235].
- [21] Italian Multicentric Study on Centenarians (IMSC) Laboratory parameters of Italian centenarians. *Arch Gerontol Geriatr* 1998;27:67–74.
- [22] Janu MR, Creasey H, Grayson DA, et al. Laboratory results in the elderly: the Sydney Older Persons Study. *Ann Clin Biochem* 2003;40(pt 3):274–9.
- [23] Kubota K, Kadomura T, Ohta K, et al. Analyses of laboratory data and establishment of reference values and intervals for healthy elderly people. *J Nutr Health Aging* 2012;16:412–6.
- [24] Ryden I, Lind L, Larsson A. Reference values of thirty-one frequently used laboratory markers for 75-year-old males and females. *Ups J Med Sci* 2012;117:264–72.
- [25] Tietz NW, Shuey DF, Wekstein DR. Laboratory values in fit aging individuals—sexagenarians through centenarians. *Clin Chem* 1992;38:1167–85.
- [26] Tsang CW, Lazarus R, Smith W, et al. Hematological indices in an older population sample: derivation of healthy reference values. *Clin Chem* 1998;44:96–101.
- [27] von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370:1453–7.
- [28] Altman DG. Construction of age-related reference centiles using absolute residuals. *Stat Med* 1993;12:917–24.
- [29] Altman DG, Chitty LS. Design and analysis of studies to derive charts of fetal size. *Ultrasound Obstet Gynecol* 1993;3:378–84.
- [30] Wright EM, Royston P. Simplified estimation of age-specific reference intervals for skewed data. *Stat Med* 1997;16:2785–803.
- [31] Risch L, Lisec I, Jutzi M, et al. Rapid, accurate and non-invasive detection of cerebrospinal fluid leakage using combined determination of beta-trace protein in secretion and serum. *Clin Chim Acta* 2005;351:169–76.
- [32] Awofeso N. In: Re-defining 'Health'. Comment on the article: Üstün BT, Jakob L. Calling a spade a spade: meaningful definitions of health conditions. *Bull World Health Organ* 2005; 83:802. Available at: http://www.who.int/bulletin/bulletin_board/83/ustun11051/en/print.html. Accessed December 23, 2016.
- [33] WHO. Constitution of the World Health Organisation. Geneva Switzerland: WHO; 2006. Available under: http://www.who.int/governance/eb/who_constitution_en.pdf. Accessed July 01, 2015.
- [34] Huber M, Knottnerus JA, Green L, et al. How should we define health? *BMJ* 2011;343:d4163.
- [35] Edvardsson M, Levander MS, Ernerudh J, et al. Clinical use of conventional reference intervals in the frail elderly. *J Eval Clin Pract* 2015;21:229–35.
- [36] Risch L, Lhotta K, Meier D, et al. The serum uromodulin level is associated with kidney function. *Clin Chem Lab Med* 2014;52:1755–61.
- [37] Sakem B, Nock C, Stanga Z, et al. Serum concentrations of 25-hydroxyvitamin D and immunoglobulins in an older Swiss cohort: results of the Senior Labor Study. *BMC Med* 2013;11:176.
- [38] Stanga Z, Nock S, Medina-Excobar P, et al. Factors other than the glomerular filtration rate that determine the serum beta-2-microglobulin level. *PLoS One* 2013;8:e72073.
- [39] Fraser CG. Inherent biological variation and reference values. *Clin Chem Lab Med* 2004;42:758–64.
- [40] Lloyd-Jones DM, Evans JC, Levy D. Hypertension in adults across the age spectrum: current outcomes and control in the community. *JAMA* 2005;294:466–72.
- [41] Kaufman DW, Kelly JP, Rosenberg L, et al. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. *JAMA* 2002;287:337–44.
- [42] Yourman LC, Lee SJ, Schonberg MA, et al. Prognostic indices for older adults: a systematic review. *JAMA* 2012;307:182–92.
- [43] Gill TM. The central role of prognosis in clinical decision making. *JAMA* 2012;307:199–200.
- [44] Zakai NA, Katz R, Hirsch C, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch Intern Med* 2005;165:2214–20.
- [45] Spivak JL. Anemia in the elderly: time for new blood in old vessels? *Arch Intern Med* 2005;165:2187–9.
- [46] Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood* 2006;107:1747–50.