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The SENIORLAB study in the quest for healthy elderly patients

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Abstract: Reference intervals (RIs) for laboratory analyses by and large, are provided by analytical platform providers – the provenience and preanalytics of materials for the calculation of intervals often remain arcane particularly relating to the age group of donors. In an observational, prospective cohort study on 1467 healthy uniracial Caucasian residents >60 years of age, 105 frequently used lab tests were done on one blood sample. With a nonrestrictive definition of health, several pathological lab results pointing to occult disease have been found and published from SENIORLAB so far. The RIs found for hemoglobin in women went from 117.9 to 152.4 g/L (80–84 years) and in men from 124.9 to 170.6 g/L (90% confidence interval [CI]). This article lists RIs computed with SENIORLAB data for such frequently ordered analyses as platelet counts, vitamin B12 and folate, ferritin and analytes measured to estimate metabolic performance in glucose turnover. In fact, 64.5% of the cohort showed prediabetic fasting plasma glucose (FPG) and/or glycated hemoglobin (HbA_{1c}); total serum folate levels but not red blood cell folate decreased with progressing age. As much as 66% of evaluable study participants had insufficient levels of 25(OH) vitamin D. Published reports from SENIORLAB are referenced in this article.

Keywords: elderly; endocrinology; hematology; reference intervals.

List of abbreviations: AADD, age adjusted D-dimer; AI, artificial intelligence; ANOVA, analysis of variance; ASH, American Society of Hematology; CCHA, Clinical

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Consortium and Healthy Ageing; CRP, C-reactive protein; CSLI, Clinical and Laboratory Standards Institute; DDG, differential diagnosis generators; DDX, electronic differential diagnoses; DPREP, disease pattern recognition engine platform; CI, confidence interval; eGFR, estimated glomerular filtration rate; FGP, fasting plasma glucose; FtH, ferritin heavy chain homopolymer; HbA_{1c}, glycated hemoglobin A1; ITP, immune thrombocytopenic purpura; NHANES, National Health and Nutrition Examination Survey; OECD, Organization for Economic Cooperation and Development; holoTC, holo transcobalamin; MMA, methyl malonic acid; ROS, reactive oxygen species; RI, reference interval; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; WAS, White Anglo-Saxon.

Introduction

Ten years ago, the SENIORLAB study was designed to establish reference intervals (RIs) of current medical lab assays suitable for validation performed in elderly patients. At the onset and upon immediate evaluation, the RIs were computed on cross-comparative data sets usually grouping ages from 60 to 69, 70 to 79 and above 79 years of age. The study was concluded with RIs of most lab analyses performed. Questionnaires were sent to surviving participants in an endeavor to relate quality of remaining life years to lab values determined at the onset of disease. Here, we report part of the results in the context of geriatric medicine.

Before this paper goes into the data obtained, let us cite Georges Canguilhem (1904–1995) [1] who tells us in his thesis on *The Normal and the Pathological*: ‘Médecins et chirurgiens ont une information clinique et utilisent aussi parfois des techniques de laboratoire qui leur permettent de savoir malades des gens qui ne se sentent pas tels’ (English translation: “With clinical information at hand, doctors and surgeons sometimes use lab techniques to identify persons who are sick without signs and symptoms”).

With the extension of the human life span the laboratory medicine community began to scrutinize RIs applicable for validating assays ordered for older patients. There

were already short lists of analytes available, the RIs of which substantially deviate from work force age groups. It has long been known that sex hormones decline and a number of parameters have joined an ever growing list, often immediately upon initial recognition of their importance in the improvement of diagnosis.

Aging, senescence and maturation are denominations with slightly different meanings. Whereas senescence is interpreted as endogenous degenerative processes leading to death, aging encompasses a wide array of passive or nonregulated, degenerative steps determined, at least in part, by exogenous factors; gradual deterioration with aging can be looked at as a fail-safe system with collateral turnover that is prone to repair [2]. Involution such as with the thymus may feign senescence and age-related decline in T cell output is a risk factor for many cancer types and infectious diseases [3], thymic atrophy which starts at puberty in all vertebrates. When we submitted the study protocol of SENIORLAB to the competent agencies back in 2008, many studies had already addressed the question of specific RIs in the elderly. The summary of the then screened literature can be listed as is shown in Table 1.

The increasing mean age of the world's population concerns all those survivors of accidental death unruffled by senescent biology. That there are differences in the causes of death among species and, ultimately among different human races and different individuals, was made evident from studies on the survivor patterns for cohorts of organisms. Pearl and Miner [4] described several possible types of survivor curves. In Figure 1, curve A is characteristic of a population in which death is predominantly caused by disaster, predation or other random external selection forces.

Table 1: Progressing age-dependent deviations of reference intervals of routine lab parameters.

Elevated	Decreased
Alkaline phosphatase	Calcium, zinc
Cholesterol	Creatine kinase, eGFR
Clotting factors VII and XIII	Dehydroepiandrosterone, testosterone
D-dimer	Estrogen
Ferritin	Growth hormone
Fibrinogen	IGF-1, interleukin-1
Postprandial glucose	Phosphor, selenium, thiamin
Parathormone	X-tocopherol (vitamin E)
Interleukin-6	Vitamins B6 and B12, vitamin C
Noradrenalin	
Parathyroid hormone	
Prostate specific antigen	
Triglycerides	
Uric acid

Figure 1: Survival curves under different conditions.

(A) Survival curve at a constant rate of mortality, i.e. 50% per unit of time elapsing. (B) Survival curve of a population that exhibits increased susceptibility to death with age. (C) Survival curve for a population with termination guided for all members by natural processes, such as senescence in the context of our topic. Note that the decrease in C is slightly less pronounced because senescence varies among individuals of the same species.

The constant rate of removal results in a survivor curve that drops exponentially, gradually approaching zero; mortality of 50% per unit of time. The nonaligned pattern in B denotes an initially strong surviving population with increased susceptibility to death with age in the absence of compromising influences of any type whereas the shape C shows a survival curve from a population with abrupt termination of all members by natural processes, like senescence and the role it plays as an endogenous control phenomenon [4]. The list of institutions who are trying to get to grips with senescence biology and its impact on lab assays is long, such as those from the Framingham Study, the Leiden Longevity cohort or the Baltimore Longitudinal Study of Aging (BLSA) and the Berlin Aging Study to name but a few are studied.

Good practice for statistics

During recent decade, many centers have scrutinized the accuracy of RIs. Providers of analyzer modules for routine lab assays release trustworthy RIs for each parameter, however, they do not always show how these results are obtained. Many of the currently used RIs for clinical laboratory assays are based on samples from younger adults and the industry shuffles files and recuses information if asked to show age ranges, race and sex of the RI providing populations. A large majority of the RIs of adults are established with repeat blood donors who have gone through detailed questionnaires on their health and

qualification to donate. Healthy elderly individuals as donors for samples to establish RIs of seniors are not so easy to recruit as most of them fail to overcome the entry obstacles. The sample size for those qualified for non-diseased conditions remain difficult to achieve as for the statistical power to be met, the experts must agree that a minimum of 120 test results for a given analysis is required – this allows for the usual coefficients of variations of the test being studied. The sample size is also limited when group partitioning and filters for evaluation must be applied such as for definite drugs taken, filtering for analytes linked to the one addressed or partitioning by sex, age, ethnicity, menstrual cycle or menopause/andropause passed [5]. Of the appropriate statistical methods to establish the RIs, once the data are produced, nested analysis of variance (ANOVA) is the likely method of choice owing to its ability to handle multiple groups and being able to adjust for multiple factors. Outliers may be identified using the Box-Cox transformation. Either RIs or decision limit (“cut-offs”) should be reported from the lab to the clinician but not both, according to a Clinical and Laboratory Standards Institute’s (CLSI) C28-A3c requirement. Age, gender distribution and association can be analyzed using the non-parametric Wilcoxon rank sum, Kolmogorov-Smirnov tests and quantile regression methods to estimate the 95th percentile involving RIs. This cornucopia of encoding parameters brings the understanding of RI in the elderly into the limelight.

The SENIORLAB study, an observational, prospective cohort of voluntary subjectively healthy residents of Switzerland aged 60 years or older could be completed from 2009 within 2 years of the onset for evaluation of RIs. The study procedure is described in detail elsewhere [6]. A total of 1467 participants were included and a fasting blood sample was drawn under optimal preanalytical conditions. A simplified comprehensive geriatric assessment

(CGA) form designed to assess the wellbeing of the participants included the following questions honestly answered (primary exclusion criteria): Drugs: do you take drugs containing steroids?, Are you under ill-adjusted antihypertensive therapy?, Do you suffer from thyroid diseases/are you substituted with thyroid hormones?, Do you have diabetes mellitus?, Have you suffered from cancer during the last 5 years?, Were you hospitalized during the month prior to enrolment and Do you abuse alcohol? In contrast to many previous studies, SENIORLAB applied a tedious but diligent search in Caucasian elderly by applying exclusion criteria through a rigorous process before including the successful women and men in the study and the loading of each age decennium approached a Gaussian distribution (Figure 2). As of the time of this writing, 127 participants have passed away. Once a candidate to enter the study was accepted her/his obstacles continued at the stage of including her/his lab results for calculating the RI of a given analyte: the outliers were excluded and a trellis-work was set up holding back those participants with pathological results on analytes likely to influence the analyte under computation for RI; C-reactive protein (CRP) >10 mg/L proved to be the most frequent analyte excluded.

More than 110 laboratory analytes were measured, and a biobank is stored on our premises.

Reference intervals found for selected analytes

Hematological and related tests

Many elderly people, in contrast to working age adults may expect mild anemia, often associated with medical comorbidities [7, 8]. Most reports in this context pertained to

Figure 2: Age distribution of participants.

A Gaussian distribution of healthy SENIORLAB study participants with respect to age becomes apparent.

residents in nursing homes or attending mobile examination centers for medical care with a variety of complaints or even with seniors constrained in a hospital leaving the reader in doubt [9, 10]. In the USA, the 3rd National Health and Nutrition Examination Survey (NHANES) study focused on racial groups of elderly patients revealing a prevalence of anemia in men and women >65 years and approximately more than 10% in Blacks than in Caucasian residents [11, 12]. In patients over 85 years, this difference exceeded 20%. Updates are available at: <https://www.cdc.gov/nchs/nhanes/index.htm>.

Hemoglobin

Physiologic concentrations of hemoglobin (Hb) in human blood range from 120 g/L (female) and 130 g/L (male) to 168 g/L (both genders) under which anemia and above which polycythemia are diagnosed. We are currently in need of reference ranges suitable for healthy older subjects and unbiased by debilitated individuals. Indeed, the defining of the lower cut-offs provided by the World Health Organization (WHO) are 120 g/L for women and 130 g/L for men (WHO Technical Report Series 1968); these cut-offs do not account for age.

The complete blood count assays of SENIORLAB were done using a SysmexTM XE-5000 (Horgen, Switzerland) hematology analyzer and clinical chemistry assays followed routine workflow on modular platforms.

Two years later, in 2013, a retrospective evaluation of the surviving participants, 764 women and 631 men with ages ranging from 60 to 99 years old and the data were explored in more detail: mean Hb and 95% confidence intervals (CIs) were calculated using logarithmic transformation in order to correct for skewness of the data. Overall, women had a mean Hb of 136 [95% confidence interval (CI) 120–155] and men had a mean Hb of 148 (95% CI 128–172). Detailed results by age groups are presented in Figure 3. The Hb level was steady over age groups until 74 years of age for men and 80 years of age for women. Interestingly, only a few subjects fell outside the WHO limits with 20 women having a Hb less than 120 (2.9%) and 20 men a Hb less than 130 (3.6%) (Table 2).

We looked for frequent causes for anemia such as iron and vitamin deficiencies (folic acid and B12). In the cohort, 37 subjects had a ferritin level lower than 20 µg/L (2.9%). Among these, nine of the 37 (24.3%) had a low Hb (WHO definition) and only one of the 37 (2.7%) had a slightly lower mean corpuscular haemoglobin concentration (MCHC). The micronutrient folic acid and vitamin B12 were also assessed (see later); 98 subjects (7.8%) had folic acid

Figure 3: SENIORLAB: median hemoglobin concentrations. Please note both gender median values approaching each other with progressing age.

Table 2: Hemoglobin measurements using the SYSMEX-5000 system.

Age, years	Female			Male		
	n	Percentile		n	Percentile	
		2.5	97.5		2.5	97.5
60–64	135	120	153	129	135	167
65–69	181	123	153	159	132	169
70–74	162	122	153	117	131	170
75–79	133	118	156	110	127	166
80–84	91	118	153	79	125	171
>85	62	114	149	37	116	160
Total n	764			631		

Please note the tendency for mild anemia in the very old healthy study participants of SENIORLAB. Data cut down to six age groups.

<10 nmol/L. Among these individuals, six of 98 (6.1%) were anemic. The definition of vitamin B12 deficiency varies but is usually accepted as lower than 200 pmol/L in elderly persons: in the whole population, 398/1255 (31.7%) subjects had vitamin B12 levels <200 pmol/L and 105/1255 (8.4%) had vitamin B12 concentrations less than 150 pmol/L. Using these two limits, these subjects were anemic in 11/398 (2.8%) and four of 105 (3.8%), respectively. The impression of a limited impact of folic acid and B12 deficiency lingers in anemia of the elderly [13].

SENIORLAB is able to define new Hb RIs in elderly subjects reporting as healthy by the strict clinical criteria in a large cohort of subjects. The results show a very limited proportion of subjects with WHO defined anemia, thus indicating that even in older subjects, the discovery of a low Hb is likely to be associated to an underlying pathology.

The most apparent change seen in bone marrow along with senescence is decreased cellularity. By the 65 years of

age, hematopoietic marrow cellularity has been estimated to recede to 30% with fat taking over. Age-related qualitative changes in hematopoietic cells are: skewed X-chromosome inactivation, telomere shortening, accumulation of mitochondrial DNA mutations and micronucleus formation; growth hormone and erythropoietin lose part of their activation potency as is suggested by the NHANES III database. The prevalence of anemia was lowest among males between 17 and 49 years (1.5%) and highest (26%) in males older than 85 years and was found to be associated with poor nutritional status in 62% of 3751 community-dwelling older people in the PolSenior study [14].

To treat such mild anemia or not depends on a diagnostic algorithm relating to its background. If this not an option then mild transfusion therapy might be prescribed but this has not gained ground, not the least because of persistent concern of transfusion-transmitted disease [15].

Platelet concentrations and clotting system

In contrast to red blood cells (RBC) with their narrow physiological window (120–155 g/L), normal platelet ranges are wide open (150–350 × 10⁹/L). Healthy individuals with a sustained platelet count between 100 × 10⁹/L and 150 × 10⁹/L have a 10-year probability of developing autoimmune disorders of 12%. Further investigation is required to establish whether this risk is higher than in the general population and whether an intensive follow-up results in an improvement of prognosis [16].

Quite a sizeable number of asymptomatic individuals with platelet counts ranging between 100 × 10⁹/L and 150 × 10⁹/L have participated in the study. However, both the clinical features of these individuals, as well as the natural history of their mild thrombocytopenia, often remain occult. Regrettably, an undetermined number of individuals may go on to develop an overt disease associated with a low platelet count, others may maintain normal or borderline platelet counts until late in their lives, but to date no consistent figures concerning these events are available.

In the elderly, immune thrombocytopenic purpura (ITP) is frequent and calls for treatment in the presence of bleeding episodes. Drug-induced thrombocytopenia must be considered in patients under poly-medications [17] which are limited to five in SENIORLAB.

The individual concentrations of platelets in the SENIORLAB cohort are on Figure 4 (n = 1424) and Table 3.

Thus, SENIORLAB evaluates the RIs of one of the most commonly ordered laboratory tests, i.e. platelet counts, in the age groups most frequently seen in clinical medicine,

Figure 4: Platelet concentrations in all participants.

Table 3: Reference intervals and median values of platelet concentrations (×10⁹/L) in the SENIORLAB study.

	Platelet concentrations, ×10 ⁹ /L	Median concentrations of platelets, ×10 ⁹ /L
95% CI 60–69 years	152–380	230
95% CI 70–79 years	135–351	228
95% CI over 80 years	128–350	223
90% CI 60–69 years	162–330	ND
90% CI 70–79 years	148–331	ND
90% CI over 80 years	140–320	ND

i.e. seniors and the single values of the elderly show gender- and age-specific RIs deviating considerably from the commonly employed RIs. It follows, that the practice of using single gender- and age-independent RIs for platelet counts (e.g. 140–350 × 10⁹/L) should be abandoned for individuals aged 60 and more [18]. In any event, related age-adapted RIs in apheresis practice and clinical routine will reduce harm.

Platelets have long been at the forefront of research activities in Bern, Switzerland. Ernst Lüscher co-discovered the thrombin receptor in these “cells” [19] and platelet participation in immunological reactions, hence their susceptibility for damage upon autoimmune triggers is now well recognized and important in clinical pictures in pediatrics and internal medicine [20, 21]. Successful treatment using i.v. immunoglobulin therapy anticipated by years the recognition of its action mechanism [22]. Treatment-naïve patients with immune thrombocytopenia should pass through a therapeutic strategy updated to most recent therapeutic acquisitions independently on mild thrombocytopenic platelet counts [21]. Geriatric patients without primary clinical evidence of thrombocytopenia close to 80 years old frequently showed mild thrombocytopenia [23]. Some researchers have attempted the production

Table 4: Reference intervals found in the SENIORLAB study presented as to be understood by an educated layman – participant of the study or not.

Analyte	Unit	Reference intervals recommended for routine	Reference intervals found in the present study	Interpretation
Cholesterol	mg/dL	5.2–6.2	3.4–8.1	Slightly higher levels allowed
Bilirubin	μmol/L	<24	<24	No difference
Creatinine	mmol/L	44–80	52–124	Slightly higher levels allowed
Glucose	mmol/L fasting	3.9–5.6		Slightly higher levels allowed
Electrolytes				No difference
TSH	mU/L	0.50–4.3	0.44–4.1	Slightly lower levels allowed
Iron	μg/L	5.4–19.0	10.0–30.0	Slightly higher levels allowed
Ferritin	mg/L	14.0–152.0	18.0–295.0	Slightly higher levels allowed
Hemoglobin	g/L	120–156	114–152	Slightly lower levels allowed

of platelets from stem cells in bioreactors <http://plateletbiogenesis.com/> such that platelet transfusions could be substituted, at least in part, by man-made platelets.

D-dimers

SENIORLAB has not addressed D-dimers which are well known to depend on age. A recently published study from the Mayo Clinic [24] used cut-off for a negative D-dimer based on reference ranges regardless of the age of the donor population is informational. These healthy American study participants, i.e. 241 subjects ranging from 21 to 91 years of age had their overall reference range determined (CLSI guideline states to partition if over 8% for either group is outside the overall reference range) baseline D-dimer increases with aging making age-adjusted D-dimer (AADD) cut-offs for patients older than 50 years mandatory. A significant association between D-dimer and age was found at the median in this study ($p < 0.0001$). For practical reasons and to set the upper cut-off, our validation office applies the multiplication of the values by 10 times the age, a 90-year-old patient can go up to 900 μg/L, whereas a 50-year-old subject should keep their D-dimer level below 500 μg/L. SENIORLAB (Table 4) has not been able to measure D-dimers but increased D-dimer values in the elderly make AADD for patients older than 50 years mandatory.

Vitamin B12 and folate

Vitamin B12 (cobalamin) was identified more than 80 years ago as the antipernicious anemia factor in liver, and its importance in human health and disease has resulted in much work on its uptake, cellular transport and utilization. Plants do not contain cobalamin because they have no cobalamin-dependent enzymes.

Deficiencies are therefore common in strict vegetarians, and in the elderly, who are susceptible to an autoimmune disorder that prevents its efficient uptake. In contrast, many algae are rich in vitamin B12, with some species, such as *Porphyra yezoensis* (Nori), containing as much cobalamin as liver [25]. The vitamin B12 and folate status in non-anaemic healthy older persons needs attention the more so as a decrease in levels may be anticipated from reduced hematinic provision and/or impaired intestinal uptake. After excluding the results of as many as 324 participants for elevated CRP, folate and/or B12 supplementation, and death after 1 year, 1143 results were available from the 637 women and 506 men with vitamin B12, holotranscobalamin (holoTC), methylmalonic acid (MMA), homocysteine (Hcy), serum folate and RBC folate [26]. Besides RI calculations, receiver operating characteristics (ROC) analysis was also done to assess the accuracy of the individual parameters in recognizing a deficient vitamin B12 status parameters. The age groups: 60–69, 70–79 and ≥80 years had median B12 (pmol/L) levels of 237, 228 and 231, respectively ($p = 0.22$), holoTC (pmol/L) of 52, 54 and 52 ($p = 0.60$) but Hcy (μmol/L) 12, 15 and 16 ($p < 0.001$), MMA (nmol/L) 207, 221 and 244 ($p < 0.001$). Hcy and MMA (both $p < 0.001$), but not holoTC ($p = 0.12$) and vitamin B12 ($p = 0.44$) were found to be affected by kidney function. Total serum folate and RBC folate drift apart with increasing age: whereas the former decreases ($p = 0.01$) RBC folate remains in the same bandwidth across all age groups ($p = 0.12$). A common RI combining age and gender strata can be obtained for vitamin B12 and holoTC, a more differentiated approach seems warranted for serum folate and RBC folate (Figures 5 and 6).

These results can be set in relation to findings recently unfolded in the framework of the BLSA which for many aspects of studies into senescence goes back as early as 1958 to trace the effects of aging. Evaluation of gait speed is

Figure 5: Vitamin B12 and holoTC serum levels in the three age groups classified according to sex. The box plot representation with vitamin B12 (A) and holoTC (B) as appearing in three age groups. The difference of vitamin B12 concentrations among males and females is statistically significant in 70–79 year old ($p=0.001$) and not significant in the participants aged 60–69 ($p=0.07$) and ≥ 80 years ($p=0.69$). The same comparisons of holoTC concentrations are significant for participants aged 60–69 and 70–79 (both $p < 0.001$), and not significant in the participants ≥ 80 years ($p=0.77$) [26]. Copyright retained by the authors as published on: <https://www.biomedcentral.com/getpublished/copyright-and-license>.

Figure 6: The uncertainty of the holoTC values is substantial. Other markers to delineate the vitamin B12 status have been quantitated with SENIORLAB and are plotted here displayed using receiver operating characteristic (ROC) curve. (Reprinted, with permission from Risch et al. [26]). Copyright retained by the authors as published on: <https://www.biomedcentral.com/getpublished/copyright-and-license>.

now considered by neurologists as a very reliable measure of functional capacity with well-documented predictive value for major health-related outcomes. Thus, the BLSA more recently, undertook measurements of B12 and other micronutrients in over 1000 male volunteers who at their entry into the study were examined for putative vitamin B12 and homocysteine associations with gait speed.

As part of the BLSA, independent associations of serum levels of vitamin B12 and plasma concentrations of homocysteine with gait speed, 774 study participants were stratified for linear regression in age categories 50–69, 70–79 and 80 years old [27]. Gait speed (m/s) was assessed using the 6-min usual pace test. Vitamin B12 and homocysteine concentrations were collected using standard clinical protocols. Elevated homocysteine concentrations were associated with a decline in gait speed after adjustment for covariates, hence confirming previous reviews on 827 participants from the BLSA (mean age 67, range 50–96 years) in which the mean erythrocyte corpuscular volume (MCV) and cognitive performance was followed over time [28].

Vitamin D

The measurements of SENIORLAB on 25(OH) vitamin D have been published in an Open Source journal and are freely accessible; the reader is invited to consult the results on the Internet [29]. In brief, 66% of the subjects had insufficient levels of 25(OH) vitamin D; severely deficient levels of 25(OH) vitamin D were found in 7.98% of the total study population. Once again, this observation can be set in the dual and very opposite perception of so many healthy elderly persons having low vitamin D levels either being healthy, hence their RIs are normal for their age or that indeed, such low vitamin D levels call for routine substitution once a retired citizen reaches an age beyond 60 years. However, vitamin D levels have been found recently to be associated with cognitive performance and verbal memory and verbal fluency [30] and it is well recognized that low vitamin D levels favor a wide number of autoimmune diseases [31]. Larger population studies are needed to resolve this point of principle. Older adults might not adequately absorb micronutrients hence substitution and lab control of sufficiency will be mandatory.

Ferritin

The importance of serum protein ferritin, in addition to its iron storage and its acute phase reactant status, has recently been put into the context of its proinflammatory activity and interaction with inflammasome generation

unknown type 2 diabetes mellitus (T2DM) among its participants [34]. The fasting plasma glucose (FPG) and glycated hemoglobin A_{1c} (HbA_{1c}) levels were used for screening. A total of 1362 subjects were selected for analysis (613 men and 749 women; age range 60–99 years). Subjects with known T2DM were excluded. Plasma glucose levels were measured by means of the hexokinase procedure, and HbA_{1c} was measured chromatographically and classified using the current American Diabetes Association (ADA) criteria whereby, for international understanding, some of us require the results in mmol/mol units. The prevalence of individuals unaware of having prediabetic FPG or HbA_{1c} levels, was 64.5% (n=878); unknown lab based T2DM was found in 8.4% (n=114). On the basis of HbA_{1c} criteria alone, significantly more subjects with unknown FPG impairment and laboratory T2DM could be identified than with the FPG alone. The prevalence of PreD as well as of T2DM increased with age thus anticipating laboratory evidence of impaired glucose metabolism. Laboratory identification of people with unknown out-of-range glucose values and overt diabetic hyperglycemia might improve the prognosis by delaying emergence of overt disease.

Senescence of kidney function

Renal function declines as we age, almost with mathematical precision: creatinine clearance, the estimated glomerular filtration rate (eGFR) as well as the number of glomeruli undergo senescence-associated regress. Kidneys are components of metabolic patterns [35] but follow their senescence on their own [36]. In addition, decreased sodium reabsorption and potassium excretion, reduced urinary concentrating capacity and alterations in the endocrine activity of the kidney add up to deterioration of renal function in the elderly. SENIORLAB study participants have been scrutinized for eGFR – related analytes, including cystatin C and uromodulin [37]. Cystatin C, a low molecular weight biomarker of kidney function is also less dependent on age, race and muscle mass, is increasingly addressed for estimation of metabolic fitness. It has been studied for its role in predicting new-onset or deteriorating cardiovascular disease. It also seems to play a role in brain disorders involving amyloid in Alzheimer's disease. In humans, all cells with a nucleus (cell core containing the DNA) produce cystatin C as a chain of 120 amino acids. With SENIORLAB study participants we have related cystatin C levels to kidney vigor in the context of a 4-year follow-up survey recording information about overall morbidity and mortality. The sysC-crea ratio was significantly higher in women and increased progressively with

age such that SENIORLAB, for the first time can reveal age- and sex-specific RIs in seniors indicating a relative retention of biologically active low-molecular weight compounds, perhaps predicting risk for overall mortality and morbidity in the elderly.

The relationship between serum uromodulin and different stages of kidney function (i.e. cystatin C-based 2012-CKD-EPI eGFR_{CysC} >90 mL/min/1.73 m², 60–89 mL/min/1.73 m², 45–59 mL/min/1.73 m² and <45 mL/min/1.73 m²) allowed us to focus on 289 participants (140 males/149 females; mean age 71±7 years) yielding significant differences in serum uromodulin among the four groups according to different kidney function stages (p<0.001). Serum uromodulin displayed inverse relationships with creatinine (r=−0.39), cystatin C (r=−0.42) and urea (r=−0.30) and, correspondingly, a positive relationship with eGFR_{CysC} (r=0.38, p<0.001 for all). Serum uromodulin levels contrast to the different conventional renal retention markers by displaying lower concentrations with decreasing kidney function. The new generation of uromodulin assays allow measurements in serum or plasma – their results complete the information of established kidney function markers; it appears that mild reductions could signal initial stages of kidney disease raising the interest of kidney transplantation centers (physiological values around 200 ng/mL). Extrarenal metabolism impacts, such as body mass index (BMI), nutrition or muscle mass do not need to be factored into the results by additional calculations, as with classic markers which make the results of SENIORLAB on uromodulin helpful to nephrologists.

A generalized overview from the point of view of study participants

A WHO Clinical Consortium on Healthy Ageing (<http://www.who.int/ageing/health-systems/clinical-consortium/en/>) has recently been set up to globally focus on clinical research by generating new evidence that disease-free survival might be possible during terminal senescence; SENIORLAB is part of such efforts in that it allows caretakers and the healthy elderly to sense upcoming health problems early on in the medical laboratory. The Clinical Consortium on Healthy Ageing (CCHA) commissioned by WHO features a multidisciplinary composition and encourages multi-institutional experts and innovators in the field of geriatric medicine to produce standards, clinical norms and guidelines, often even understood by the educated layman.

SENIORLAB takes advantage of an increased recognition of ethical guidelines. Is it not delicate to contact family members of deceased participants and to ask them for the cause of death? We have experienced upset in telephone calls aimed at feeding the data set of SENIORLAB with additional information on the fate of participants during the last 8 years or so.

In fact, we wish to:

- (i) identify associations between different parameters,
- (ii) identify the power of lab assays to diagnose certain circumstances,
- (iii) identify the prevalence of occult disease in subjectively healthy individuals, and
- (iv) identify the prognostic factors for the investigated outcomes, including length of survival.

Such analysis would identify a given analyte as a biomarker. Along the whole SENIORLAB evolution those involved in communicating with study participants have gone through a learning process on how to tell lab results to apparently healthy seniors. The few outliers we found were immediately communicated to the participant and to her/his medic such that a second assay on a subsequent blood sampling was performed for confirmation/disconfirmation under medical surveillance.

The participant was less frightened if an analysis could be situated into the context of senescence as is the case with slightly elevated HbA_{1c} or CRP levels now confirmed for a discrete w/v increment in seniors. In the PolSenior study, performed on over 4000 seniors over 65 years old, the IL-6 and CRP levels were higher in aging-related diseases/disabilities and lower in successfully aging individuals and higher IL-6 and CRP levels were associated with poorer physical performance [38]. Progressing glycation in advanced glycation end products is considered as non-enzymatic modifications of proteins or lipids upon physiological exposure to sugars, occasionally accumulating in tissue [39].

RIs found in the SENIORLAB study ought to be understood by anybody interested in the field, including educated laymen – participants of the study or not. An ever increasing community of biologists, now estimated to add up to 2 million researchers worldwide, follow, observe and understand the message and bring them over to others. The skills likely to be in greater demand include interpersonal skills, higher cognitive skills and systems skills, all of them potentiating the usage of artificial intelligence (AI). Not only medicine proper but basic biological topics such as embryogenesis, maturation and senescence will be absorbed by such portals such as, e.g. www.ibm.com/Watson and others.

To understand how diseases arise an extraordinary amount of information needs to come together; quick and inexpensive sequencing DNA and RNA now explores all the “omes”: genome, the full set of genes, transcriptome, the RNA made from the genes, the metabolome: small molecules, such as sugars, fatty acids and amino acids, involved or generated by cellular processes and the fluxome: metabolic reactions whose rates can vary under different conditions. The Human Protein Atlas (<https://www.proteinatlas.org/>) includes a high-resolution map of the locations of more than 12,000 proteins. Data-driven medicine undoubtedly improves health care, we have come a long way since Carl von Rokitansky (1804–1878) gave medicine a firm scientific basis by introducing correlations between symptoms and the diseases that cause them.

Enrolment of older people with hematological disorders into clinical studies still lags behind. This age group remains underrepresented in blood cancer clinical trials, according to a study presented at the 2017 Annual Meeting of the American Society of Hematology (ASH) by experienced Dr. Bindu Kanapuru, a hematologist in Falls Church, VA, USA (<http://www.ascp.com/News/58356>). Age alone must not be an exclusion criterion. With only a few patients aged 75 years or older, critical information on the safety and effectiveness of new therapies is at loss which also pertains to the value of medical laboratory assays if age-specific RIs are at stake. The magnitude of the disparity is particularly concerning given that the number of adults aged ≥75 years who are diagnosed with hematological malignancies is expected to rise as the population ages. Currently, one out of five patients diagnosed with blood cancers is aged 75 years and older [40] <http://www.hematology.org/Newsroom/Press-Releases/2017/8067.aspx>.

Obviously, pharmaceutical companies are reluctant to include these older patients in phase III clinical trials, because of the uncertainty on how they will tolerate investigational medications and because of approaching death. This population is also extremely heterogeneous. We can encounter one 75-year-old individual who is very healthy whilst another person of the same age is frail and has a lot of coexisting illnesses.

Conclusions

Separate RIs for age groups – how far must we go?

Along with maturation and puberty, RIs change within in relatively short periods of time from newborns to

toddlers, to child, puberty and adolescence. Their computation is tedious because healthy youngsters are not easily to bleed and one needs parental consent. Analytes like CRP are <10 mg/L up to 10 days of age, thereafter they are <5 mg/L as in adults, or folic acid which is 14–51 nmol/L up to 1 year of age – thereafter 3–35, or free fatty acids which are 0.5–1.6 between 1 and 12 months, then are 0.2–1.1 nmol/L, from 7 to 12 years; these are examples which vary substantially with progressing age. Impressively, copper (Cu) amounts to 1.4–7.2 $\mu\text{mol/L}$ the first 5 days of life and thereafter, and certainly after age 1 year, Cu in plasma goes as high as 11.9–30 $\mu\text{mol/L}$ in healthy children – Cu being an essential microelement found in all living organisms with the unique ability to adopt two different redox states – in the oxidized (Cu^{2+}) and reduced (Cu^+) [41, 42].

Open questions

The SENIORLAB study remains active with some questions waiting for an answer about using RIs for the validation of lab results

- (i) Definition of health. Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (Official Records of WHO, no. 2, p. 100, 1948). Depending on the field of interest, health needs to be defined in a broad sense such as those of being sound in body, mind or spirit. Various online news sources reflect current usage of the term “health” as a wide array. Some authors have argued that older people are less study-oriented than younger people, lack the skills to participate or do not want to be actively involved in research and developing services [43].
- (ii) The finding of intervals which deviate from RIs in use for work-force age groups comes as little surprise with certain analytes. Thus, the slightly lower median hemoglobin concentrations or the slightly higher CRP and HbA_{1c} concentrations in the elderly reflect nothing but the biochemistry of senescence. These findings are very far away from the finding of 25(OH) vitamin D concentrations in the low ranges, now confirmed by other authors to occur in the elderly [44].
- (iii) Whether to take into consideration or forget about specific RIs of the elderly for validation purposes is a still a debated contrasting juxtaposition [45]. There are pros and cons. Gender partitioning with sex related hormones is now clearly an issue, since SENIORLAB reveals sex differences in certain hormones, none the least in cortisol. Conversely, substitution of

low vitamins B12 or vitamin D likely prolongs survival, contributes to quality of remaining life expectancy.

- (iv) Chronobiological aspects with nyctohemeral opal variance of results are well known for cortisol, peaking in the morning, as for plasminogen activator inhibitor and this has been outlined in detail elsewhere [46].

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