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Modifications in hemoglobin levels associated with age in an outpatient population from northern italy

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A reduction in hemoglobin levels is common in older subjects. The objective of this study was to investigate the association between changes in blood counts and age in a large outpatient population of adult subjects, in order to verify to what extent such changes may be considered physiological.We examined blood count results in the province of Modena (Italy) from January 2010 to August 2022. Data were analyzed with the platform Anaconda 3, Python 3.7. Appropriate hemoglobin data were extracted from 4,676,003 samples. Hemoglobin levels in subjects over 75 years were largely below lower limits for both sexes (49.3% of 509,834 exams and 35.4% of 704,343 exams for males and females, respectively). The trend was similar in relation to single values per person per year. To exclude patients with some major systemic diseases, we limited our observation to subjects with normal values of serum glucose, creatinine, and alanine transaminase (ALT). In this set of 822,166 analyses, a clinically relevant proportion of older males (nearly 30%) still had hemoglobin values below normal. Such trend was apparent in older age strata. Our findings suggest caution in the interpretation of blood counts in older patients. We therefore advocate a tailored approach in this population.

Valid interpretation of laboratory data is crucial to proper clinical decisions. In particular, blood count is widely used as a disease marker in routine investigations.

Older subjects often present with reduced red blood cells, hematocrit and hemoglobin levels.

According to the World Health Organization (WHO), the presence of hemoglobin levels ≤ 12.0 g/dl in women and ≤ 13.0 g/dl in men supports anemia diagnoses, regardless of age[1]. However, these criteria have subsequently been questioned[2,3]. Indeed, an appropriate definition of standard medical reference limits should consider an array of aspects (age, gender, ethnicity), among which age may play a central role.

Based on the above thresholds, diagnosis of anemia can be suggested for a relatively high number of older subjects, particularly males. More importantly, it can lead to subsequent clinical workups and significant concerns in terms of subjective perceptions of a compromised health condition.

It should be acknowledged that anemia represents a clinically relevant and prevalent health problem for older people worldwide. In spite of the variable findings reported in the literature, its prevalence may be as high as nearly 50% in male subjects and 40% in females[4,5]. Furthermore, its presence may be underestimated, particularly in countries at high risk of nutritional deficiencies. In many instances, nonetheless, a reduction in blood count indexes could be considered a physiological age-related phenomenon, which may not require specific diagnostic or therapeutic intervention.

The causes leading to a reduction in hemoglobin and red blood cells in older subjects are numerous[6–8]: (1) deficiency of iron, folate, or vitamin B12 is relatively common in this age range and is often present in a context of global malnutrition; (2) chronic kidney disease and chronic inflammatory conditions; and (3) hematological malignancies present in old age. Even by excluding common etiology of pathological blood count alterations, however, "unexplained" anemia is still present in one-sixth to one-third of total anemia cases, according to the literature[4,9,10].

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The etiology of unexplained anemia potentially may include multifactorial causes: (1) alterations to bone marrow density and function, (2) reduced erythropoietin response, (3) minor and asymptomatic endocrine dysfunctions, (4) subclinical chronic inflammatory conditions, and/or (5) unrecognized or minor nutritional deficiencies[4,10].

Recognition of patients with unexplained age-related reduction in hemoglobin who do not require further investigation or treatment may be difficult. Recognition requires well-balanced clinical judgement along with expertise in the management of health problems in older people. On the other hand, extensive clinical engagement under conditions that appear physiological or para-physiological might be considered unjustified. More importantly, it appears hardly sustainable in the epidemiological context of global population aging and in terms of healthcare resource allocation.

Data mining may prove useful in this regard. Analysis of extended data sets provided by laboratories collecting huge population samples ("big data") can allow for a precise investigation of blood count parameters across different age strata and subsequent characterization of age-related changes. An accurate interpretation of laboratory results might ultimately lead to economic benefits for healthcare systems and patients alike.

The purpose of the present work was to investigate the relationship between aging and blood count parameters in a large population of outpatients undergoing biochemical evaluation in a Northern Italian tertiary care hospital laboratory. A potential secondary objective could be a redefinition of optimal cutoff values for the diagnosis of anemia in older subjects.

Methods

Patient population and data source

We examined all blood count results between January 2010 and August 2022. These had been collected by laboratories outside hospitals in the province of Modena (Italy). Results were analyzed in the central laboratory of the City Hospital of Baggiovara (Modena, Italy), a tertiary care referral hospital. This set includes about 99% of laboratory examinations in the area, which consists of nearly 700,000 inhabitants. The laboratory is accredited and certified by national healthcare institutions.

The analysis was confined to subjects older than 25 years. No further specific inclusion or exclusion criteria were adopted. However, samples from hospitalized patients were not considered in this study.

Hemoglobin levels, red and white blood cell and platelet counts (RBC, WBC, and PLT, respectively), and mean corpuscular values (MCV) were analyzed.

During the initial phases of the project, the medical datasets described below were preliminarily analyzed to develop and validate a data mining algorithm for advanced autonomous medical monitoring and diagnostics. More specifically, the laboratory data were analyzed against reference intervals and used to determine "normal" or "out of range" values.

Ethical statement

All procedures were performed in accordance with ethical standards from the Helsinki Declaration of 1975, as revised in 2013. The study protocol was approved by the local Institutional Review Board ([IRB] Ethics Committee Area Vasta Emilia Nord, Modena section). Informed consent was waived by virtue of the retrospective design of the study and pseudonymous data collection at source.

Representation of the data and statistical analysis

All test results of hemoglobin, WBC, RBC, and PLT counts, and MCV were extracted from a data set of *Order Groups* performed in the healthcare district (AUSL-MO) and extracted from an overall data base, including approximately 87,000,000 laboratory test results from approximately 700,000 patients.

Overall, hematological records of adult subjects over 25 included 4,673,529 *Order Groups*, in which each order group corresponds to a set of *Orders* prescribed by doctors to the same patient at the same time. Order groups are composed of more tests or *Work Orders* intended as elementary laboratory analysis generating a single result. According to our study design, a hematological *order group* (namely, the complete blood cell count) always consisted of a mix of hematological *single work orders*, basically hemoglobin, RBC, WBC, and PLT counts, and MCV. A total number of 1,931,623 Order Groups (each consisting of a single work order for hemoglobin, RBC, WBC, and PLT counts, and MCV) were available for male patients, and 2,741,906 for female patients.

The analysis of each test (a *single work order*) was specifically designed to characterize value distribution according to the following criteria: (1) age range (25–34, 35–44, 45–54, 55–64, 65–74, 75–84, 85–94 and older than 95 years); (2) gender (male or female); (3) inpatient/outpatient status; and (4) mean and standard deviation. Furthermore, a boxplot analysis (a work tool that enables graphical representation of a set of measures) based on the same age range for patients was conducted. This highlighted quartiles as shown in Supplemental Fig. 1.

Data were stored in a Vertica Sequel Server. The analysis was performed by means of Anaconda 3, a widely used science platforming addition to Python 3.7, and related statistical and graphical packages such as Pandas, Numpy, Seaborn and Matplotlib. The specific functions and libraries utilized for every statistical variable are detailed in Supplemental Table 1.

Because people with lower blood counts are likely to have their blood tests repeated more frequently than people with normal values, a potential source of bias might be present due to a higher number of values within the same period of time. To minimize such risk, a single value was considered for each subject per year, corresponding to the average value for that parameter in the year.

Furthermore, an attempt was made to extract "healthy" people from the overall population in the absence of more detailed clinical information. To perform such an extraction, we selected subjects whose laboratory tests (including glucose, creatinine and alanine transaminase (ALT) as indicators of particularly common diseases) tended to be within normal ranges. Standard reference intervals for the laboratory were considered. These

comprised glucose 70–110 mg/dl, creatinine 0.50–1.40 mg/dl, and ALT 1–40 U/L for all age ranges. By adopting the same criteria used for the entire population, we subsequently only considered the mean blood count value per year in this subset of patients.

Results

The database included 62,796,583 different samples for the parameters used in the present investigation for the resident population in Modena from January 2010 until August 2022. When errors about gender, age and personal code were discarded, complete blood count data samples in subjects over age 25 amounted to 4,673,529.

Hemoglobin data were extracted from 4,676,003 samples, obtained from 340,344 female and 281,043 male subjects. On average, every patient underwent 7.51 tests over the 12-year period. Figure 1 shows the distribution of hemoglobin levels, subdivided by gender and age range, around the median. Supplemental Table 2 details the number of tests performed in male and female subjects in every year of observation, along with distribution across different age ranges in the first and last years of observation.

As shown in Fig. 1, panel A, hemoglobin levels were below the lower limit of the normal range in a large share of samples from older subjects: 49.3% of 509,834 samples in males and 35.4% of 704,343 samples in females, for subjects aged 75 years and older. Such a trend appeared to be more marked in the older age strata under investigation, i.e. 85–94 and older than 95.

To minimize selection bias and in light of repeated blood work for some patients in the same year, an additional analysis was performed. For this purpose, data were divided by year and with a view to the average value per person per year. As shown in panel B, a similar trend was observed, indicating that 40.5% of men and 27.5% of women over age 75 were still below normal limits.

To exclude patients with systemic or organ disease(s), furthermore, we limited our observation to subjects with normal values of serum glucose, creatinine and ALT, again considering a single value per patient per year (Fig. 2). In this set, composed of 824,043 samples, 29.7% of male subjects over age 75 still showed values below normal. In females in the same age interval, the median value appears to be lower, but most values (82.5%) fall within the normal range. Here, as in the previous illustration, the alterations described are more prominent with advancing age.

Figure 3 illustrates RBC distribution in the study population. Panel A shows data for the whole population and entire data set, whereas the average value per year is reported in panel B. Once again, a large share of observations for male subjects older than 75 (more than 50% in the whole data set and nearly 50% considering the mean value per year) fell below the normal range.

In older female subjects, the median value was reduced, but the box was very close to or within the normal range.

The distribution of RBC concentration in the "healthy" population (subjects with overall normal values of serum glucose, creatinine and ALT) is shown in Fig. 4. A similar trend was found to be present with nearly 40% of values below the lower normal limit in males older than 75.

When looking at most MCV values, in contrast, the entire boxes fall within the normal range for both the whole population data set (data not shown) and average values per year in the population with normal glucose, creatinine and ALT vales (shown in Fig. 5, panel A).

In theory, normal median values of RBC volume in our population might be detected in the presence of a bimodal distribution of this variable. This could be due to the coexistence of subjects with larger corpuscular volume (due to folate and/or vitamin B12 deficiency) and patients with iron deficiency-associated microcytosis. To exclude this possibility, a violin plot analysis was conducted. Evidence of bimodal distribution was detected neither in the whole population (data not shown) nor after excluding abnormal glucose, creatinine and ALT values, as illustrated inFig. 5, panel B. The impact of iron, folate and vitamin B12 levels was not investigated because this type of investigation would have restricted data analysis to a large extent.

Finally, the analysis of WBC and PLT concentrations showed consistently normal values across all age strata for both genders, in the whole data set (data not shown) and in the evaluation of average yearly values limited to the "healthy" population. However, a trend toward lower PLT concentration was observed in male subjects (Fig. 6, panels A and B, respectively).

Discussion

Blood cell counts may constitute the most common set of laboratory tests requested by physicians in the context of routine laboratory evaluation.

In older subjects, blood count alterations such as reductions in hemoglobin and red blood cell concentration are extremely frequent, while the prevalence of anemia is consistently higher compared with younger age groups[11,12].

In these patients, anemia is associated with increased morbidity, mortality and, in general, impaired health conditions[13–19].

In most situations in which anemia is present, underlying causes are well recognized and, in most instances, treatable. In a number of cases, nonetheless, etiology cannot be clearly defined. This leads to diagnosis of "unexplained anemia of aging", which may likewise deserve adequate consideration and management to prevent adverse outcomes[10,20].

The likelihood of a reduction in blood count parameters being regarded as a physiological change associated with aging should be considered. From this perspective, the thresholds defining the presence of anemia, derived from the reference ranges established by WHO criteria, might need to be reconsidered.

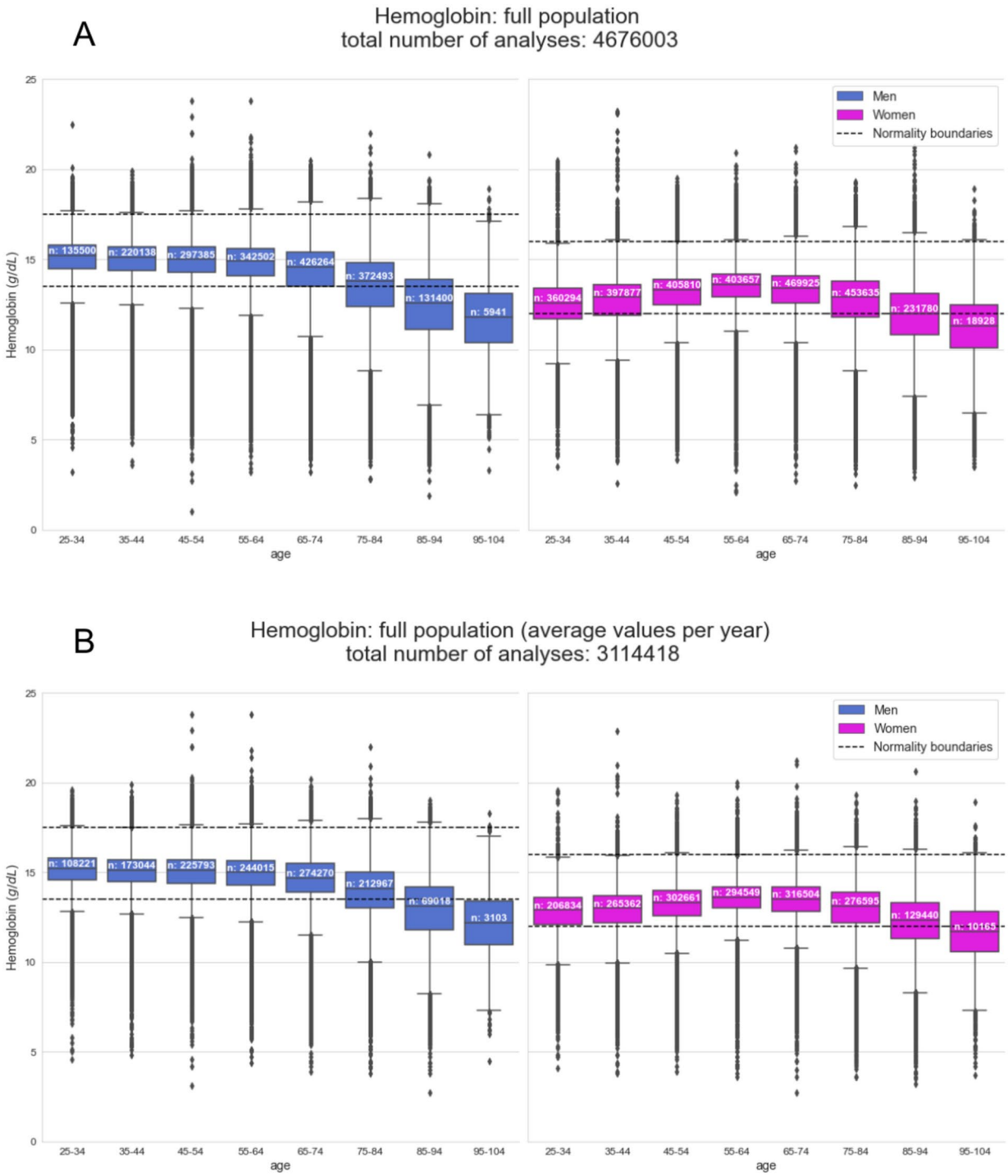

Fig. 1. Distribution of hemoglobin levels, subdivided by gender and age range. Panel **A**: whole data set (sample number: 4,673,529). Panel **B**: average value per person per year (sample number: 3,112,541).

Awareness that in some instances, a reduction in blood count indexes does not necessarily require specific diagnostic or therapeutic interventions may have relevant implications for worldwide population aging and from the perspective of proper resource allocation.

The data mining approach adopted in the present work may prove useful for analyzing such blood count changes and allow for a precise characterization of age-related changes in blood count parameters.

We utilized a large sample of over five million blood count examinations, which is therefore highly representative of the general population living in this geographical area. Restricting the analysis to outpatients allowed for the collection of a relatively homogeneous data set.

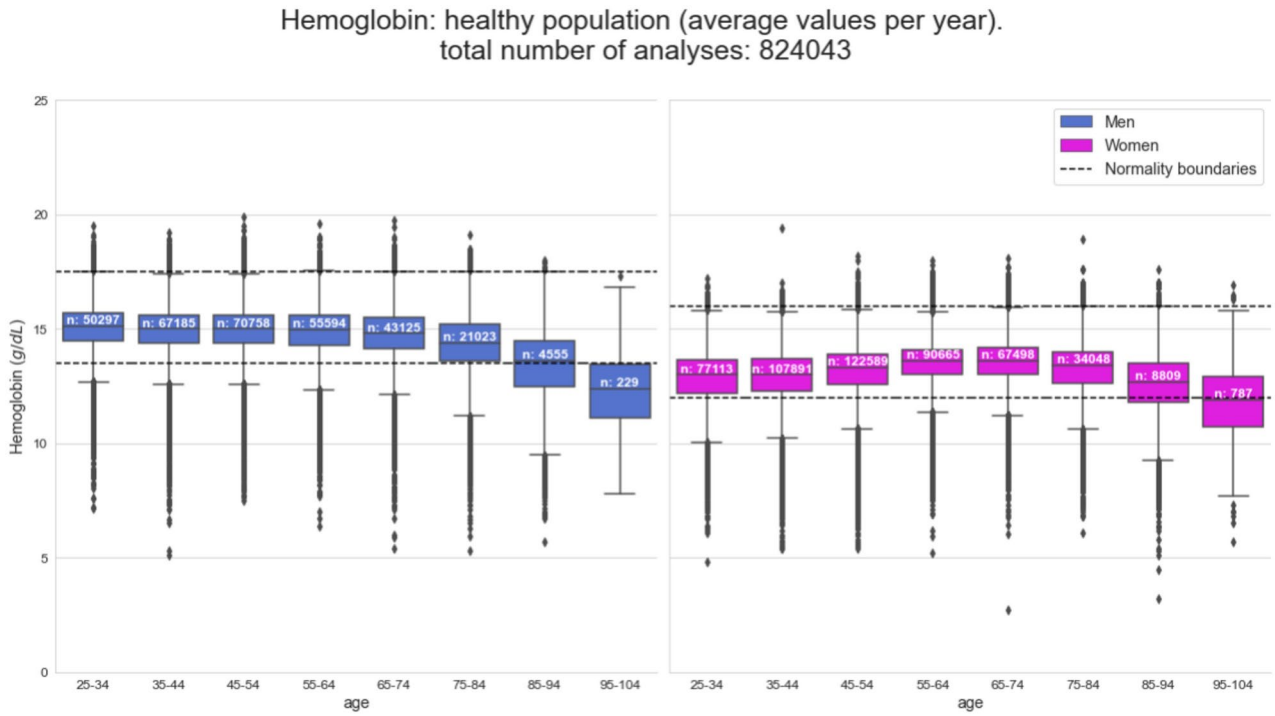

Fig. 2. Distribution of hemoglobin levels, subdivided by gender and age range in subjects with normal values of serum glucose, creatinine and ALT. A single value per person per year was considered. Sample number: 822,166.

Blood count indexes were analyzed after subdivision by gender and age strata (25–34, 35–44, 45–54, 55–64, 65–74, 75–84, 85–94 and older than 94). A decline in both hemoglobin concentration and RBC was observed, starting from age 75 and with further decreases in older age strata. In many societies, this threshold is considered a realistic limit to defining old age even if the value is set as 65 in many countries and consistent with WHO standards.

As a consequence of this threshold, the prevalence of anemia according to WHO classification criteria increased in this group, particularly in male subjects. The finding was also confirmed when the average value per year was considered, thus overcoming the potential unbalancing bias due to multiple sampling by select subsets of patients. This finding is in keeping with previous evidence from the literature that reports a decline in hemoglobin concentration with aging[11,21–23], particularly in "older old" subjects (over age 75) compared to "younger old" ones (between 65 and 75 years). This trend was also observed in the Italian population[11,14]. The literature is consistent in showing a higher prevalence of anemia in males compared with females, which may be due to different thresholds set by WHO criteria.

Such gender-related differences were not so evident in terms of other blood parameters, although a trend toward reduced platelet concentration was observed in male subjects. The finding that this deviation from the normal range was more prominent with hemoglobin levels seems to suggest a rather selective deficit in the bone marrow, in keeping with the literature[24]. MCVs also tended to remain within normal limits over all age quartiles. Such findings are in partial contrast with previous reports[25,26], which documented a trend toward an age-related increase for this index. However, a degree of variability was found in different studies. In the present work, the violin-plot elaboration clearly excluded a bimodal distribution of this parameter. This type of distribution apparently rejects the hypothesis of different deficiencies in the study populations.

Potential causes for a "physiological" decline in hemoglobin concentration might involve a reduction in bone marrow cell mass with a corresponding increase in adipose tissue; this process may be related to a decrease in estrogen production that occurs with aging[21,27].

Changes in hematopoietic response have been described in relation to aging[28–30] and, possibly, reduced hormonal stimulation[31].

Evidence of the role of erythropoietin is conflicting[32–34]. The fact that a reduction in hemoglobin concentration was observed even in a population with normal serum creatinine seems to rule out major variations in erythropoietin levels due to impaired kidney function. However, it does not exclude a reduced response by the bone marrow of older subjects.

Subclinical endocrinological dysfunction, in particular hypogonadism, might also have a role in affecting erythropoiesis[35].

Minor and unrecognized micronutrient deficiencies and initial myelodysplastic syndromes have also been evoked as a cause of otherwise unexplained reduction in blood cell parameters[10]. In such cases, a coexistence

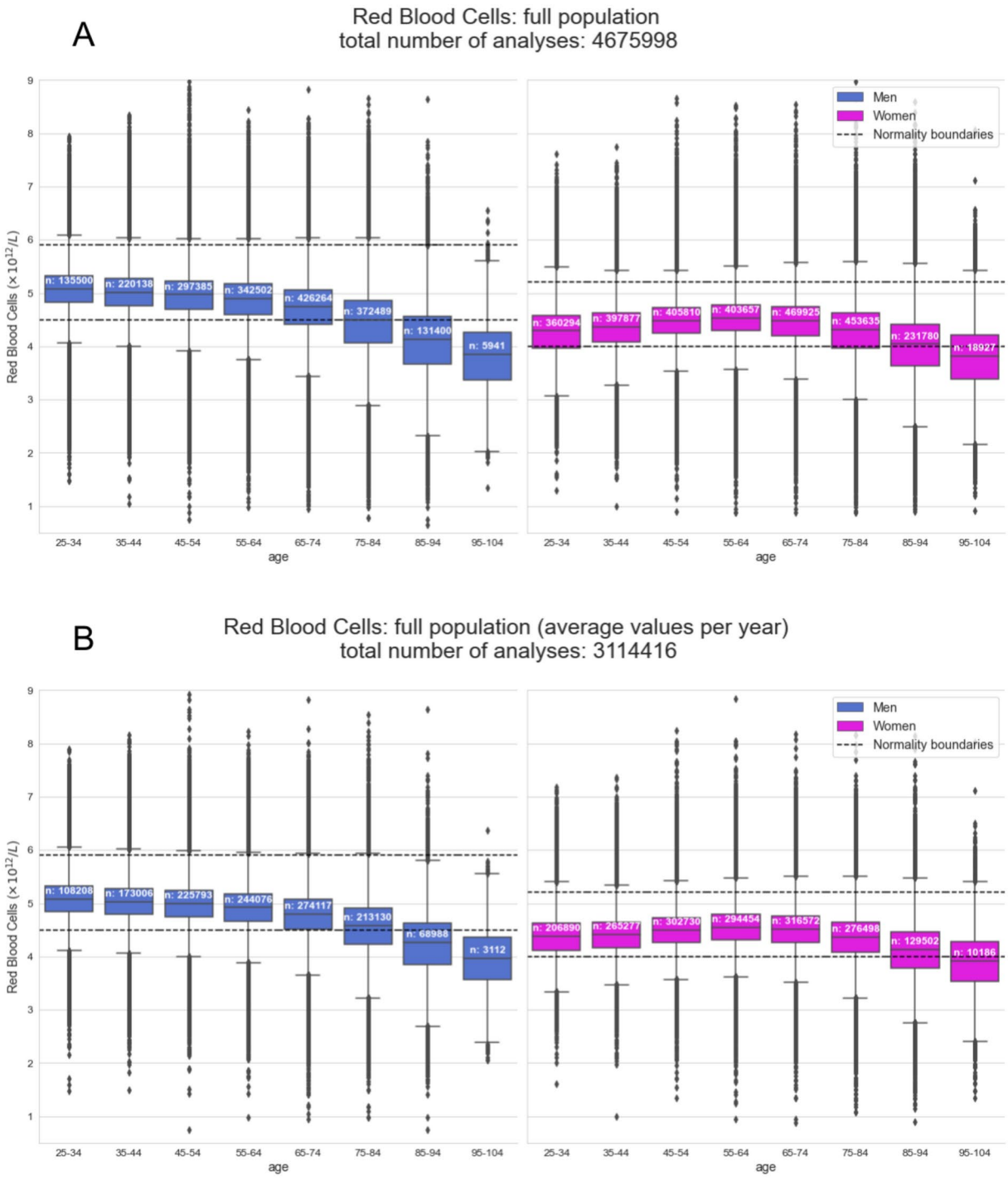

Fig. 3. Distribution of red blood cell concentration, subdivided by gender and age range. Panel **A**: whole data set (sample number: 4,673,524). Panel **B**: a single value per person per year (sample number: 3,112,539).

of low-grade inflammation with subclinical iron deficiency and reduced erythropoietin production and/or function might be established[36].

In a separate analysis, we chose to limit our observation to one sample per subject per year, consistent with other reports (26), as subjects with suspected health problems are more likely to undergo repeated blood sampling. The findings from this analysis are consistent with the whole data set (panel A versus panel B in Figs. 1 and 3, respectively). However, we should acknowledge that such an approach might somehow influence the generalizability of findings to populations with varying testing frequencies.

The study design and large quantity of data, along with their pseudonymous collection, do not allow for the acquisition of detailed clinical information. This lack of detailed clinical information represents an obvious

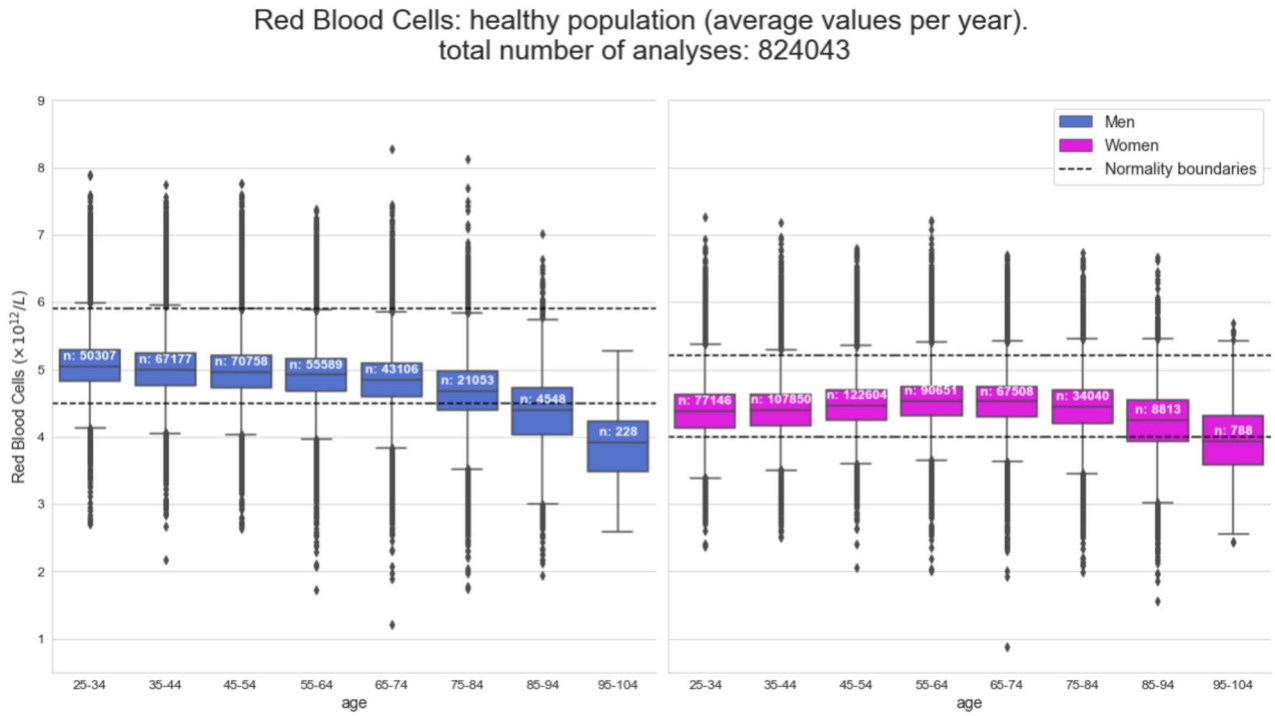

Fig. 4. Distribution of red blood cell concentration, subdivided by gender and age range in subjects with normal values of serum glucose, creatinine and ALT. A single value per person per year was considered. Sample number: 822,166.

limitation to a comprehensive understanding of the subjects' health conditions and possible hematological disorders, or specific factors that may have influenced their blood counts.

To refine our observations, we performed a sub-analysis limited to subjects with blood glucose, creatinine and ALT levels within normal laboratory ranges. We acknowledge that these markers alone may not capture all aspects of disease. Moreover, this selection may be regarded as arbitrary. In our view, however, this, along with the exclusion of hospitalized subjects, is the only feasible proposition for investigating a relatively healthy cohort of subjects in the absence of clinical data.

Regardless of potential underlying causes, which are likely to be multifactorial, awareness that in many subjects a mild reduction in hemoglobin levels can be present without overt disease states or nutritional deficiencies deserves careful consideration.

Consistent with this view, several reports in the literature have suggested reconsidering normal laboratory ranges and defining age-related reference values[21,25,26,37,38]. Preliminary analyses from this laboratory appear to be consistent with this view (data not shown).

Nonetheless, we acknowledge that redefining cutoff values for anemia in older subjects would require a comprehensive understanding of the factors contributing to changes in blood counts and an amount of clinical information that was not available. Without this clinical information, solely relying on age-related data might not provide a complete picture.

In this context, the need for a more articulate definition of normal laboratory ranges in an era of personalized medicine is in our opinion mandatory, particularly in light of the epidemiological transition toward global population aging.

Among the strengths of the present work is the analysis of a very large size of the sample. This allowed for the stratification of a relatively "normal" population of subjects. The fact that the whole dataset was limited to outpatients and excluded data from hospitalized patients with medical issues clearly reinforces this view. In our opinion, furthermore, the single-center design of the present study ensures homogeneity in data interpretation.

On the other hand, the fact that our population was confined to a local context (the province of Modena) might limit the generalizability of our findings. However, we believe that this cohort can be considered highly representative of industrialized Western societies.

A major limitation of this study is the lack of detailed clinical information inherent the "data mining" design of the study, clearly not compatible with precise clinical characterization. A number of biochemical variables (blood glucose, creatinine and ALT) were utilized as indicators of the absence of some major systemic diseases, in the absence of complete clinical data. We acknowledge that this definition of a "healthy" condition can be considered arbitrary. Furthermore, medication and the use of gastric protectors may entail the risk of bleeding and subsequently affect hematological variables. This type of situation, too, cannot be evaluated through the present approach.

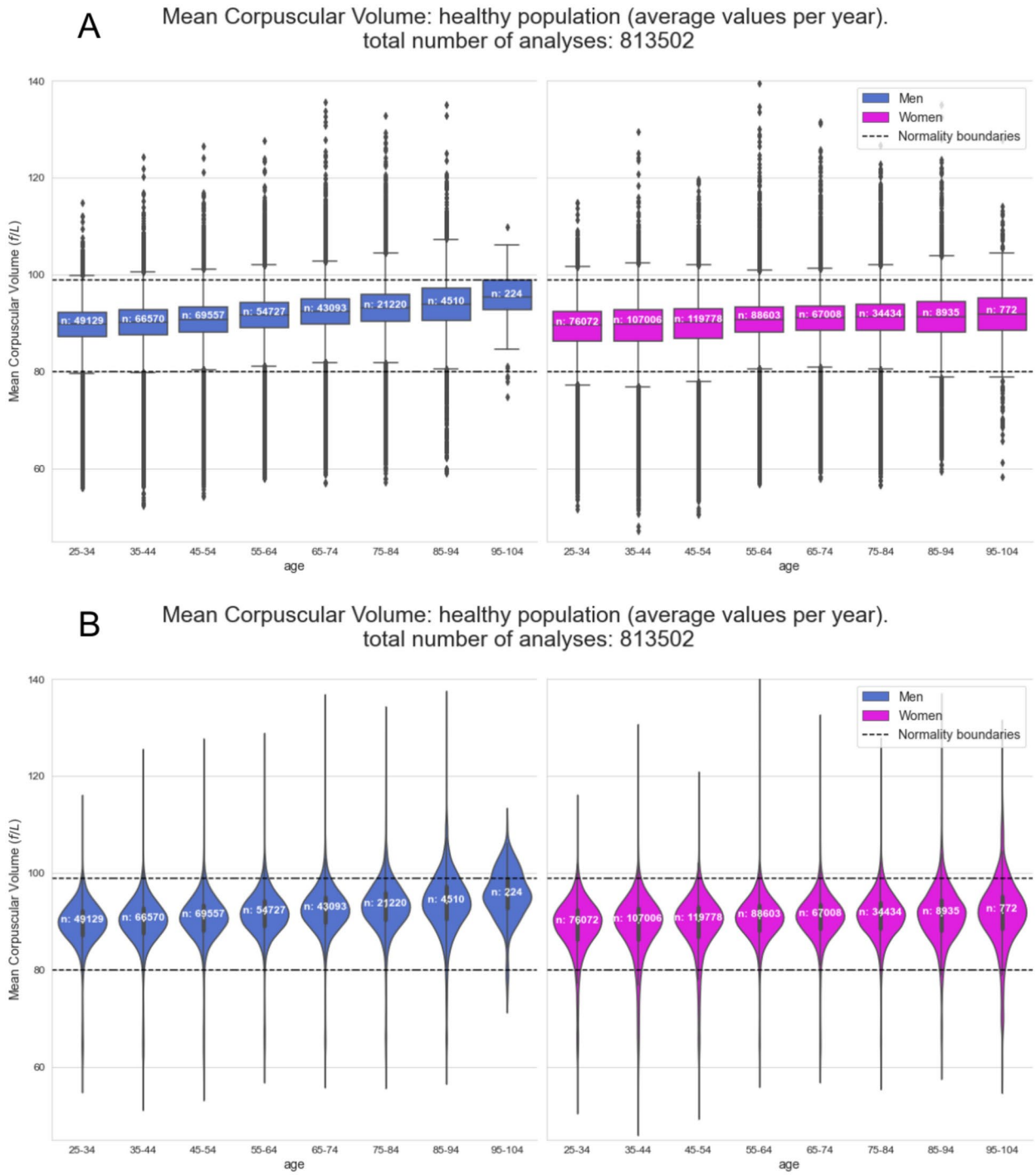

Fig. 5. Panel **A**: Distribution of mean corpuscular volume (MCV), subdivided by gender and age range in subjects with normal values of serum glucose, creatinine and ALT. A single value per person per year was considered. Panel **B**: Violin plot analysis of MCV in the same data set as described in Panel A. Sample number: 811,638.

In conclusion, the present findings strongly support the view that at least in some situations, reduced blood count indexes (in particular, reduced hemoglobin levels) may be regarded as a normal, physiological age-associated process. This finding may be relevant to accurate clinical evaluation by physicians. More than anything, it may facilitate an appropriate clinical approach to older patients and proper resource allocation with a view to a tailored approach. Needless to say, this does not minimize the need for a thorough search for potentially treatable causes of anemia and an adequate follow up of these conditions.

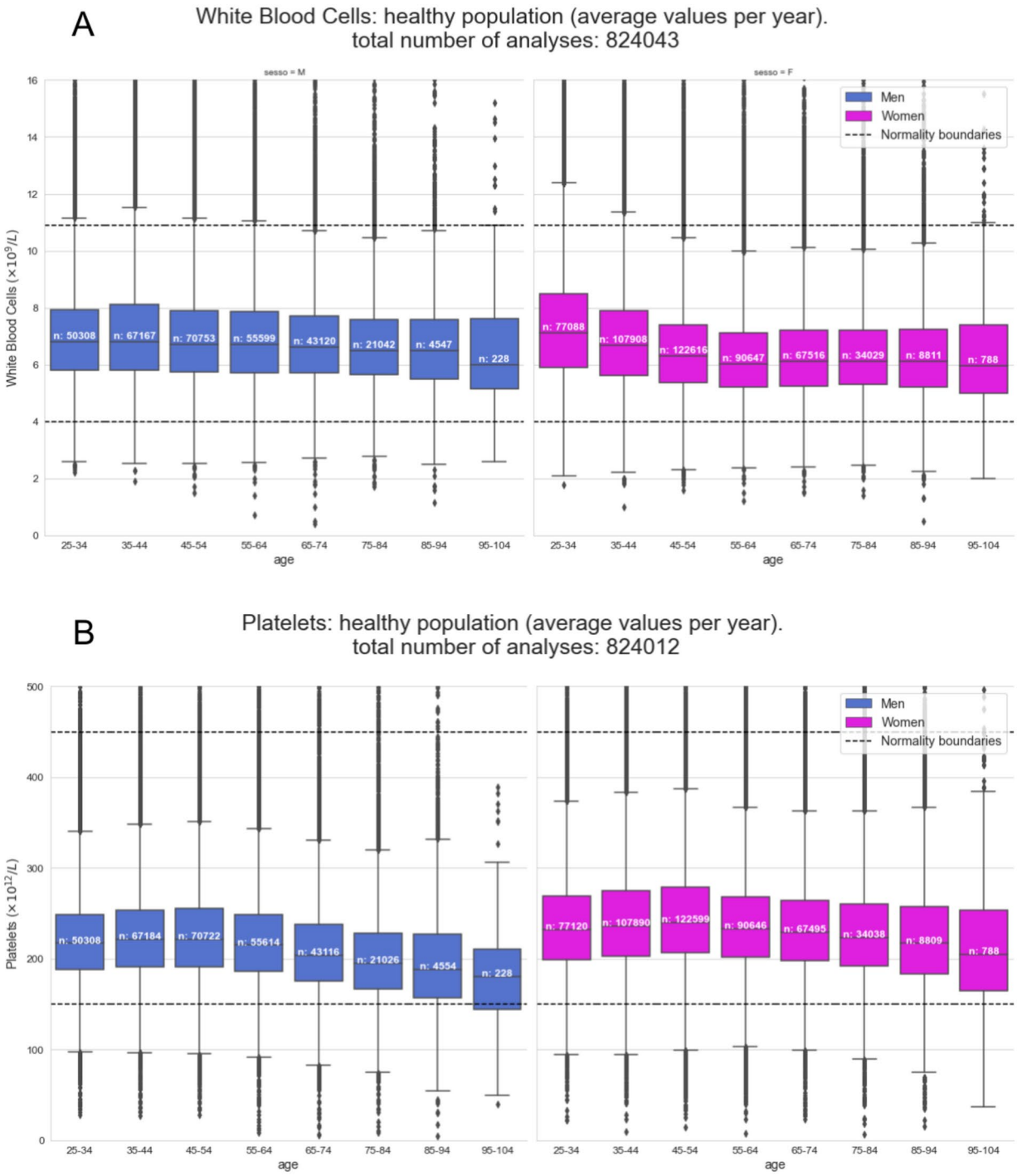

Fig. 6. Distribution of white blood cells (WBC) (panel **A**) and platelet (panel **B**) concentration, subdivided by gender and age range in subjects with normal values of serum glucose, creatinine and ALT. A single value per person per year was considered. Sample number: 822,167.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
MB, TT and CM designed the overall structure of the paper. TP, MC and TT provided thorough insight into the methodological aspects of the work. GICT and GL analyzed the interpretation of the experimental data in the light of published evidence. MB and CM drafted the preliminary version of the paper. All Authors contributed to the revision of the manuscript, and accepted its final version.

Declarations

Competing interests
The authors declare no competing interests.

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