Reference Values for Neutrophil to Lymphocyte Ratio (NLR), a Biomarker of Cardiovascular Risk, According to Age and Sex in a Latin American Population

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Background

Over the last decade, an increasing amount of scientific literature has shown the useful role of the Neutrophil-to-Lymphocyte Ratio (NLR) (represented by the absolute value of neutrophils divided by the absolute value of lymphocytes) as a predictor in the identification and prognosis of the cardiovascular disease (CVD).[1-2] Higher NLR levels are associated with a strong and positive predictive value for poor prognosis and increased morbidity in several conditions, including CVD or heart failure,[3-5] in which immunity and inflammation plays an important role. In addition, the NLR has been studied in diabetes,[6] oncologic disease (specially colon cancer),[7-11] kidney chronic disease.[12,13] Moreover NLR is associated with increased mortality risk for post cardiac events.[14,15] Recently, reports have also shown NLR to be a predictor for the development of hypertension in normotensive patients.[16]

Considering the immune-inflammatory basis of the atherosclerotic process,[17,18] it is reasonable, to consider the role of NLR as representing the balance between innate and adaptive immune responses as well as inflammatory and immunologic stages of host responses against a stressor.[19,20] However, in spite of the increased interest in the use of this biomarker,

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there are still limited data describing the NLR distribution and cut-off points in defined populations. Recently, Forget et al[21] has published "a normal value" (reference) of NLR, considering a single value for all the subjects from a small study sample (n = 413). Before, Azab et al[22], analyzed a larger cohort (9427 participants from the National Health and Nutrition Examination Survey database), but focused mainly on data of racial and/or ethnic disparities between Hispanic, non-Hispanic black, and non-Hispanic white subjects.

In 2016, we evaluated the association between NLR and arterial stiffness, represented by the pulse wave velocity (PWV), in a population-based sample of 237 adults, who were consecutively evaluated for cardiovascular tests in the context of a primary prevention program. The recruited subjects were invited to provide data about their lifestyle and cardiovascular history by completing a validated self-administered survey (WHO STEPS Survey-STEPS) as part of a major study from the cohort of the OPTIMO STUDY.[23] In that study we noted that NLR demonstrated a linear positive and independent relationship with PWV, adjusted for smoking, diabetes, dyslipidemia, and hypertension.[24] Despite reaffirming the association of NLR with arteriosclerosis with PWV as surrogate marker of cardiovascular risk, the lack of reference values limits its application on the clinical practice.

Furthermore, according to the current literature, there should exist many variations in the inflammatory response along the different stages of life, even between sexes.[25]

Hypothesis

Based on these observations, our research *aim* was to determine the NLR distribution in a large population of nonhospitalized individuals in order to provide a reference rank of its distribution categorized by age and sex.

Methods

We performed a retrospective analysis of consecutive blood samples results (n = 71,873) obtained from the database of a large central diagnostic laboratory in Buenos Aires, Argentine, during 3 consecutive months of 2015. Blood specimen analyses were ordered by general physicians and other specialists during out-patient visits. The central laboratory recruited blood samples from 22 different smaller satellite laboratories in the larger metropolitan area of Buenos Aires.

We included all cases with complete data regarding age, sex, and white blood cell (WBC) counts, including determination of neutrophil and lymphocyte counts.

Blood samples were collected in EDTA tubes (Becton Dickinson) and analyzed in 3-models LH 750 hematology analyzer (Beckman Coulter). Internal quality controls of third opinion (Bio Rad) were performed, including monthly verification procedures of analytical bias. Complete WBC counts, including neutrophil and lymphocyte counts, were measured and expressed as $\times$ 1000 cells/mm^3. NLR was calculated as the absolute value of neutrophils divided by the absolute value of lymphocytes.

Statistical Analyses

Baseline characteristics are presented as means and standard deviations, or median and interquartile range for continuous variables, and counts and proportions for categorical variables. Differences between continuous variables and categorical variables were tested with unpaired Student's *t* tests and Chi-square tests, respectively. Variables with non-normal distribution were log-transformed before comparison.

We observed measures of variability or dispersion to describe the tendency and distribution of the NLR. As measures of central tendency: mean and median; while measures of variability and spread include the standard deviation, minimum and maximum values of the variables (rank). We classified the population stratified for sex through age decades, when percentiles of normal distribution were obtained. We stratified for decades of life (16-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89, and >90 years).

Statistical analyses were performed using Statistix 7.0 (Analytical Software, Fl).

Results

A total of 71,873 subjects were included in the analyses (mean age 50.4, range 16-103 years; 66.1% females). Median of NLR varied from 1.52 to 2.44 (**Fig 1**) while in Table 1 the distribution is shown according to age and sex. A direct positive association between age and NLR values was observed in men showing a continuing increment across life (**Fig 2**). Similar relationship was found in women although a transient period of higher values was identified from age 30 to 49 years, which can be represented as a peak in the NLR evolution, suggesting a bimodal curve (**Fig 3**). The behavior of each of the NLR components was evaluated identifying that the progressive increase of NLR was mainly related to

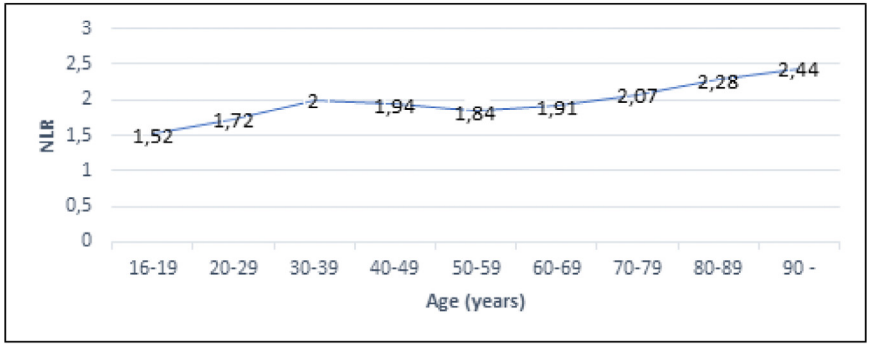

FIG 1. Median of NLR according age. NLR, neutrophil lymphocyte ratio.

variation in neutrophils and lymphocyte absolute count along life in both gender (**Fig 4**).

Conclusions

The main result of this observational study is the establishment of reference values of NLR based of an extensive data from 71,873 subjects in a large population from Argentine. To our knowledge this is the first report of NLR distribution in a large population database presented by age and sex.

NLR has several attractive characteristics, and it may be obtained from a complete blood count, and is therefore widely available, inexpensive, and easy to perform. Despite significant evidence for NLR as a useful biomarker in different clinical conditions, a lack of cut-off point and normal values constitutes a limitation for its wider use in daily practice. In our study, the NLR average value that we noted was 2.21 (median 1.91) being similar to the value reported by Azab et al[26] based on a *post hoc* analysis of the National Health and Nutrition Examination Survey population among self-identified Hispanic and non-Hispanic white subjects. Forget et al[21] in a small sample of active young adults provided a lower NLR average of 1.65 (rank 0.78-3.53). The strength of our study resides in the wide age range, including subjects from 16 to more than 90 years from only 2 main ethnic groups (self-identified Hispanic and non-Hispanic White). On the other hand, non-Hispanic black population and not significant, being less than 0.37% of the total population according to the last national data collect in Argentina.[27] According to the size of population samples, our data confirm that ageing is a strong determinant of the NLR. While the absolute values of neutrophil increase in the different decades of life, the absolute values of lymphocyte decrease (Fig 4), resulting in an

TABLE 1. Distribution of NLR according age and gender in the general population (n = 71,873)

Age (years)	n	NLR		Percentiles		Women				Men			
		Average	Median	P25	P75	n	Median	P25	P75	n	Median	P25	P75
16-19	2550	1.75	1.52	1.19	2.00	1568	1.54	1.19	2.00	982	1.50	1.19	2.00
20-29	8122	2.00	1.72	1.29	2.36	6041	1.76	1.29	2.47	2081	1.65	1.30	2.12
30-39	13,175	2.34	2.00	1.50	2.83	10,178	2.12	1.55	3.04	2997	1.71	1.35	2.22
40-49	13,156	2.17	1.94	1.52	2.52	9114	2.00	1.56	2.60	4042	1.83	1.44	2.33
50-59	11,557	2.04	1.84	1.43	2.37	7064	1.75	1.37	2.27	4493	1.96	1.55	2.50
60-69	10,861	2.20	1.91	1.47	2.53	6390	1.80	1.39	2.33	4471	2.10	1.60	2.75
70-79	8282	2.43	2.07	1.56	2.80	4672	1.95	1.47	2.63	3610	2.25	1.71	3.00
80-89	3615	2.70	2.28	1.67	3.20	2127	2.21	1.61	3.02	1488	2.43	1.75	3.38
90-	555	2.94	2.44	1.75	3.44	385	2.38	1.66	3.35	170	2.58	1.97	3.69
	71,873	2.21	1.92	1.46	2.58								

NLR, neutrophil lymphocyte ratio.

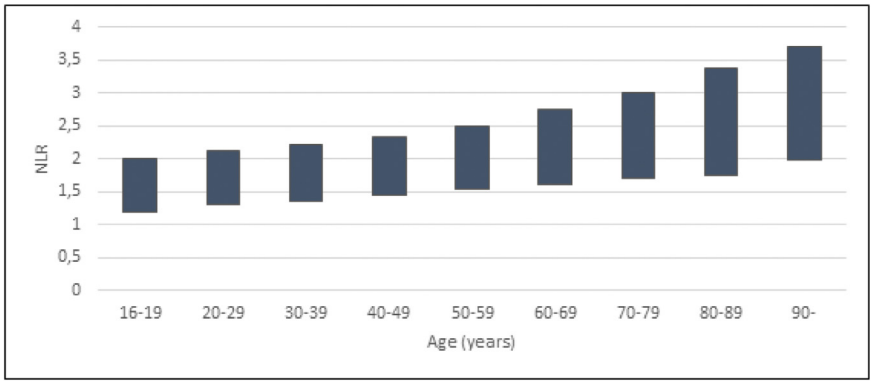

FIG 2. Neutrophil lymphocyte ratio (NLR) distribution in men (25-75th percentiles). Boxplot showing the NLR distribution according to age in men. The top and bottom lines in each box correspond to the 75th (top quartile), and 25th (bottom quartile), respectively. It shows the gradual and constant increase of NLR during the different decades of life.

increased value of NLR. This finding has important clinical implications. For example, immune-competent WBC populations play an important role in the systemic inflammatory response to infection, increasing the number of circulating neutrophils while lymphocyte counts decrease[28] during an acute event. On the other hand, it has been noted by Sawako Kato that NLR may be useful for identifying subclinical inflammation.[29] Chronic inflammation is a well-accepted biomarker that intersects and influences both psychological well-being and health-seeking behaviors.[30,31]

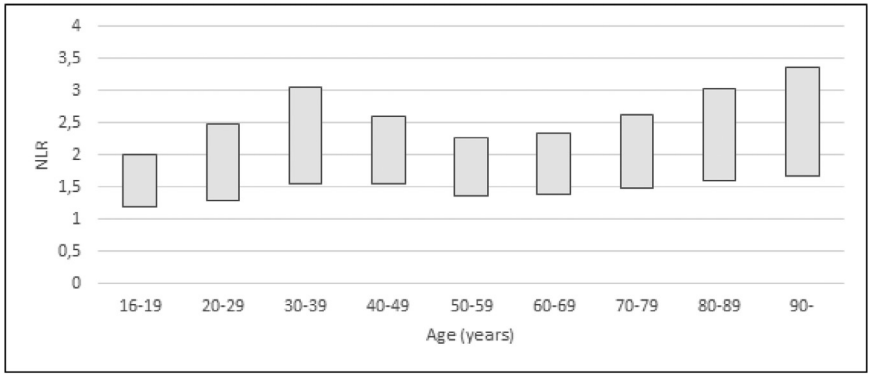

FIG 3. Neutrophil lymphocyte ratio (NLR) distribution in women (25-75th percentiles). Boxplot showing the NLR , distribution according to age in women. The top and bottom lines in each box correspond to the 75th (top quartile), and 25th (bottom quartile), respectively. In this picture we can see the peak of NLR levels during the 20th and 39th mentioned in the text, to continue increasing gradually in the rest of the decades.

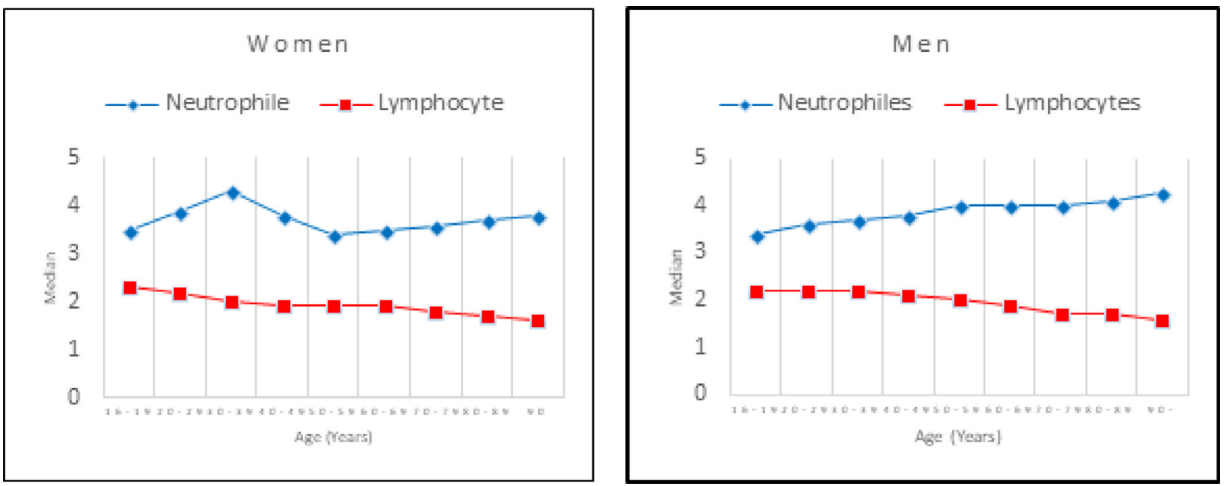

FIG 4. Median of neutrophils and lymphocytes absolutes values in men and women according to age. The red line shows the continuous degree of lymphocytes in both gender during the life, but with different levels of neutrophil (blue line), which explains the variations of NLR between men and women. (Color version of figure is available online.)

We know that in older persons there are some changes in specific components in the immune functions, due to among other things, the thymus involution,[32] changes in T cell subsets,[33] and the shortening of telomere length.[34] These variations in the absolute values are already well described. However, these mechanisms are not clearly established, older adults who suffer from chronic diseases (eg, diabetes, chronic obstructive pulmonary disease, or heart failure) are more susceptible to common infections.[35] This coincides with an impaired response to treatment or to subnormal generation of antibodies by means of vaccination,[36] a condition described as "*immunosenescence.*"[37] In consideration of this decline in the functions of the immune system, there may be associated a greater incidence of oncologic[38] or autoimmune pathologies.[39] Therefore, the findings of this study could contribute to better understanding of the immune-inflammatory nature of the ageing process.

We also report a second relevant finding, which affects the distribution of normal values, and it is the significant difference between sex. Previous studies, have not taken into account simultaneously the effects of age and sex. Even more, when the NLR role was analyzed by Sawako Kato in renal chronic disease[13] or by Neeraj Shah as utilized to predict coronary heart disease mortality,[40] neither mentioned differences between men or women. Therefore, we designed our study to investigate different NLR patterns across the life course in both sexes. In addition, we noted the significant peak in women in their 30s. This probably could be related to the fertile period and influence of sex hormones, but we admit that a limitation of our work to explain this finding is the absence of information about pregnancy or breast-feeding condition, use of contraceptives pills, corticoid therapy, drugs, autoimmune diseases, or others conditions that could modify the NLR.

On the other hand, our study has particular strengths, such as the size of the sample, the relatively homogenous demographics characteristics of the area of study as well as known in the wider Buenos Aires area, and lastly, all the tests were analyzed by 3 equal counters with the same calibration and quality assurance avoiding bias.

Another point to consider in our study is variations of NLR in the different decades of life, such as 1.52 in the 20s to 2.44 in the 90s. Some authors have established a cut-off point to detect a cardiovascular risk increase greater than 4.71 like Horne,[41] or 4.5 to be significantly associated with risk of coronary heart disease mortality by Shaha.[40] In other study, Isaac associated NLR levels greater than 3 with higher mortality among inpatients with multiple chronic conditions.[42] However, based on

our findings, we support the construction of tables according to sex and age, and not a single value as cut-off point for the general population.

Limitations of these results include the absence of data describing population baseline characteristics or medical records reflecting the prevalence of cardiovascular risk factors, use of medications, or other data on subject clinical parameters. To reduce the impact of these issues, the sample cohort could constitute enough support for the rationale of considering these values as representative, thereby perhaps compensating for the study limitations mentioned.

In summary, NLR was described according to age and sex. We consider NLR to have a potential role as a biomarker of disease risk based on its unique feasibility characteristics. These data support the need for further study and potentially wider use in clinical practice and research.

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