October 10, 2017

PharmacologyOnLine

Special issue • 2017 • vol.1 • 22-33

MONOCYTE TO LYMPHOCYTE BLOOD RATIO IN TUBERCULOSIS AND HIV PATIENTS: COMPARATIVE ANALYSIS, PRELIMINARY DATA

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Abstract

Recent data confirmed the hypothesis suggested by historical studies that the ratio of peripheral blood monocytes to lymphocytes (M/L) is associated with the risk of tuberculosis (TB) disease. We retrospectively analyzed the electronic health records of tuberculosis and HIV-positive patients who had followed day-care programs at the AIDS Center of the University of Palermo, Italy.

261 patients were recruited and divided into 6 groups as follows: healthy control group (HCG: 47 pts), latent HIV negative infected TB group (LIG, 43 pts), active HIV negative tuberculosis (TAG: 61 pts), treated tuberculosis HIV negative (TTG: 44 pts), HIV drug-naive patients tested TST and QFT-IT-negative with negative chest x-Ray (HIVnG: 44 pts), and HIV-tuberculosis coinfection (HIVTB-G: 22 pts). For each group, absolute lymphocyte (L), monocyte (M) and M/L ratio by peripheral blood was calculated.

The mean value of monocytes in the TAG group was significant, the highest (0.70±0.37 $1x10^3/\mu l$) in comparison to HGC (0.70>0.44, p-value <0.05), HIVnG (0.70>0.40, p-value <0.05) and HIVTB-G (0.70>0.45, p-value<0.05). Monocyte to lymphocyte blood RATIO showed a significant difference between groups (p-value <0.001). In particular, the mean score of M/L ratio was higher in the TAG group compared to the HGC (0.49>0.27, p-value<0.05), LIG (0.49>0.29, p-value<0.05), TTG (0.49>0.32) and HIVTB-G groups (0.49>0.27, p-value<0.05).

Our data confirm a significant difference in monocyte to lymphocyte blood ratio in tuberculosis disease. These data may be useful for monitoring and revising implementation plans for the different phases of tuberculosis disease (latent Mycobacterium tuberculosis (MTB) infection versus TB active disease).

Regarding HIV samples, the small sample size is somewhat offset by the need, fully satisfied in our sample, to enlist specific patients such as co-infected HIV/TBC who voluntarily submit to clinical trials in our geographical area.

Keywords: tuberculosis, monocyte to lymphocyte blood RATIO, HIV infection**,** co-infected HIV/TBC

Introduction

The proportion of reported TB case notifications increased from 22% in 2008 to 63% in 2013 [1,2]. Over the last decade, the number of tuberculosis cases (TBCs) in foreign-born persons has more than doubled, and the percentage of total cases is approximately 50%. This is probably because of an ongoing influx of refugees from other countries, which can contribute to the diffusion in Italy of a non-autochthonous microorganism [3-5].

In the same period, the distribution of cases reported to the Ministry of Health by age class and "non-Italian citizens" shows that the highest number of cases occurs in the intermediate age classes, with a peak in the 25-34 group which, moreover, is the most represented among non-Italian citizens present in our country. Resistance among different species of microorganism to antimicrobial drugs has developed into a public health concern [6-8, 35-40,43].

Among these microorganisms, we find TB. Multidrug-resistant (MDR) TB has increased from 2008 to 2013, with 77.4% of strains isolated from migrants from 41 countries [8-10,50].

Finally, TB/HIV co-infection is a substantial problem in the EU/EEA. The occurrence of TB in HIV-positive cases and the low TB treatment success rate indicate that TB control efforts need to address the migrant population [11,12].

Recent studies focus on the prognostic value of lymphocytes, monocytes and calculated ratios in cancer and infected patients [12-15]. There is increasingly compelling evidence that natural killer (NK) cells play a crucial role in host defense against viral infection [44-46]. Monocyte to lymphocyte ratio might also identify children who are highly protected and those who are refractory to protection with some vaccination [41,42].

In view of recently published data on flow cytometry and phenotyping analysis regarding patients enrolled in our geographical area [12], we started the investigation to analyze blood cell count of lymphocytes, monocytes and monocyte/lymphocyte ratio in Italian and migrant HIV-infected and uninfected patients.

Materials and Methods

The study participants were 261 Caucasian HIV negative and HIV-infected patients, consecutively enrolled between January 2016 and January 2017, who were being followed-up prospectively at the Department of Infectious Diseases, University Hospital of Palermo, Italy. Demographic and HIV disease characteristics were recorded and assessed as previously reported [16,17].

Full Blood Counts

Leucocyte differential counts were performed at the "P. Giaccone" University Hospital, Pathology Laboratory, University of Palermo, Italy, an accredited clinical laboratory, using standard procedures on a Sysmex automated haematology analyser.

ML ratio was calculated as the quotient of absolute monocyte and lymphocyte counts.

We have defined as affected by active tuberculosis all patients with Mycobacterium tuberculosis complex (MTBC) isolated from biological samples, as previously reported [18-21].

Patients with microbiological diagnosis underwent tuberculosis treatment, according to guidelines [22].

We identified the following groups of patients:

1. Healthy control group (HCG): 47 HIV negative individuals who tested TST and QFT-IT-negative.
2. Latent HIV negative infected TB group (LIG): 43 HIV negative patients who reported household or equivalent close contact with smear-positive pulmonary TB patients in the previous 3 months, QFT-IT-positive, with negative chest x-ray results for active pulmonary lesions and no prior preventive therapy administered.
3. Active HIV negative tuberculosis (TAG): 61 patients with a microbiological diagnosis positive for Mycobacterium tuberculosis complex (53 with pulmonary tuberculosis and 7 with extrapulmonary tuberculosis).

4. Treated tuberculosis HIV negative (TTG): 44 cured TB patients who showed a previous microbiological diagnosis.
5. HIV drug-naive patients (HIVnG): 44 patients (mean of CD4 + = 300,59 mm^3), tested TST and QFT-IT-negative with negative chest x-ray.
6. HIV-tuberculosis co-infection (HIVTB-G): 22 patients with TB diagnosis after two months of specific anti TB therapy (mean of CD4 = 70 ± 43,5 mm^3).

Patient medical records were collected and entered into a hospital medical record database. Informed consent, including consent to the publication of patient details, was signed by all individuals before they accessed the day health care services, in accordance with the Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed by Matlab statistical toolbox version 2008 (MathWorks, Natick, MA, USA) for Windows at 32 bit.

Data are presented as number and percentage for categorical variables and continuous data were expressed as mean ± standard deviation (SD), unless otherwise specified.

The multiple comparison chi-square test was used to define significant differences among percentages: if chi-square test was positive (p-value less than 0.05) then residual analysis with the Z-test to locate the highest or lowest significant presence was performed.

Multi-comparison tests on continuous data were performed with one-way ANOVA test to evaluate significant differences among means. If the ANOVA test was positive, Scheffé's method was performed for pairwise comparison. Finally only a p-value < 0.05 was considered as significant.

Results

In Table 1, we report the characteristics and multivariate analysis of the enrolled population by patient group: gender, age, country of origin (Italian/Migrant), lymphocyte and monocyte absolute blood count and monocyte to lymphocyte blood ratio.

We found a significant difference among groups for gender (M) (p-value < 0.0001): in particular, the HCG group had fewer males than any other (40.43%, p-value < 0.05).

As for age, no significant difference was found among groups (p-value = 0.207).

Regarding country of origin, there were significant differences between the groups (p-value <0.0001): in particular, there were more Italian patients in the HCG group (100%, p-value <0.05) and fewer in the TAG (22.95%, p- value <0.05) and TTG groups (22.73%, p-value <0.05).

As far as lymphocytes are concerned, there was a significant difference among groups (p-value<0.001): in particular, mean value of lymphocytes was significantly greater in the LIG group than in TAG (2.22 >1.70) ($1x10^3/\mu l$) and HIVnG (2.22 >1.45) ($1x10^3/\mu l$).

As for monocytes, there was a significant difference between the groups (p-value <0.001): in particular, the mean value of monocytes was significantly higher in the TAG group than in the HCG group (0.70 > 0.44) ($1x10^3/\mu l$), HIVnG (0.70>0.40) ($1x10^3/\mu l$) and HIVTB-G (0.70>0.45) ($1x10^3/\mu l$).

Finally, monocyte to lymphocyte blood RATIO (M/L) showed a significant difference between groups (p-value<0.001): in particular, the mean score of M/L ratio was higher in TAG than in the HCG (0.49>0.27), LIG (0.49>0.29), TTG (0.49>0.32) and HIVTB-G groups (0.49> 0.27). In Figures 1 and 2 we show the mean value of lymphocytes, monocytes and monocytes/lymphocytes for each group.

Discussion

Tuberculosis is a leading cause of death worldwide despite the availability of effective chemotherapy for over 60 years. Although Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccination protects against tuberculosis complications, such as tuberculosis of the central nervous system [23,24], its efficacy is suboptimal and under debate in cancer patients and in

pregnant women [25,47]. Particularly, recent studies show a connection between preexisting tuberculosis and lung cancer [27,28]. In addition, a correlation between breast carcinoma and previous repeated fluoroscopies in the treatment of patients with tuberculosis has been considered [32]. Therefore in these cases more efficient new treatments and diagnostic procedures should be considered, especially for young people or patients with lung cancer [29-31,33,34].

In this study, we found that lymphocyte absolute count was lower in patients with active tuberculosis than in other uninfected HIV patients, and monocyte absolute count was higher in patients with active tuberculosis than in other uninfected HIV patients.

Regarding the statistical analysis, uninfected active tuberculosis patients had a significantly lower lymphocyte absolute count compared to latent infected patients; regarding monocytes, uninfected active tuberculosis patients had a significantly higher absolute count than the healthy subjects. These findings are in accordance with data we published previously [13,16].

HIV+ patients are at increased risk of contracting tuberculosis disease despite ARTs and number of CD4+ cells [48]. Disseminated infection by rapidly growing mycobacteria like M. abscessus subsp. bolletii in patients affected by idiopathic CD4+ T lymphocytopenia have been described [49]. HIV infection is characterized by a reduction in total white cell count (WCC), neutropenia and a particular reduction in $CD4^+$ T cells but an increase in $CD8^+$ T cells. In our study we found the lowest absolute lymphocyte cell count in ART naïve patients. We presume that this data is related to the underlying HIV infection.

Regarding monocyte absolute cell count, we did not find any significant difference between HIV negative and HIV positive patients, except for subjects with active tuberculosis.

Naranbhai et al. showed that in the HIV population the ratio of monocytes and lymphocytes (ML ratio) in peripheral blood is associated with tuberculosis. Our median monocyte count was 0.4, like the 50^{th} percentile reported by Naranbhai [16] in HIV patients who developed tuberculosis during follow-up. Similar data regarding absolute monocyte count were reported by Jun Wang for the Brazilian tuberculosis population [26].

In conclusion, our preliminary results confirm the evidence demonstrated in the in vivo and vitro study on immunocompetent patients with tuberculosis [13-16,26].

On the other hand, our study raises doubts about the role of M/L ratio in subjects in the HIV population, in naïve patients especially. To better outline its role as a tuberculosis biomarker for this group of patients, a larger number of enrolled subjects is needed.

The authors encourage future research to evaluate the "gray phase" of inflammatory response against *Mycobacterium tuberculosis*. In fact, the latent and reactivation phase remains a difficult step in patient management, especially HIV positive and cancer patients.

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Table 1. Characteristics for every group with multivariate analysis

Variable	HCG	LIG	TAG	TTG	HIVnG	HIVTB-G	Multivariate analysis: p-value
Patients	47	43	61	44	44	22	
Gender (M)	40.43%	76.74%	81.97%	81.82%	84.09%	77.27%	<0.0001 (C) * HCG *** (Z)
Age	42.98±13.53	44.23±17.25	37.18±18.60	38.27±18.32	40.52±11.33	42.27±9.62	0.207 (A)
NIP	100.00%	55.81%	22.95%	22.73%	63.64%	36.36%	<0.0001 (C) * HCG ** (Z) TAG *** (Z) TTG *** (Z)
LC	1.75±0.45	2.22±0.25	1.67±0.64	1.90±0.65	1.46±0.89	2.01±1.15	< 0.001 (A) * LIG > TAG * (Sc) LIG > HIVnG * (Sc)
MC	0.44±0.21	0.54±0.25	0.70±0.37	0.54±0.25	0.40±0.31	0.45±0.22	< 0.001 (A) * TAG >HCG * (Sc) GTA >HIVnG * (Sc) TAG >HIVTB-G * (Sc)
M/L	0.27±0.16	0.29±0.22	0.49±0.40	0.32±0.16	0.33±0.20	0.27±0.19	< 0.001 (A) * TAG > HCG * (Sc) TAG > LIG* (Sc) TAG > TTG * (Sc) TAG > HIVTB-G* (Sc)

HCG = healthy control group; LIG = Latent HIV negative Infected TB group; TAG = HIV negative active tuberculosis group; TTG = treated HIV negative group; HIVnG = HIV Naïve group; HIVTB-G = Co-infected HIV-tuberculosis group; NIP = Italian nationality patients; LC = lymphocytes; MC = monocytes; A = one way ANOVA test; C = multi-comparison chi square test; Z = Z-test; * = significant test; * * = significant most frequent; *** = significant less frequent; Sc = Scheffé's test (it was performed with significant level less than 0.05); MC/LC = monocyte/lymphocyte ratio; lymphocyte and monocyte values were expressed in $1x10^3/\mu l$

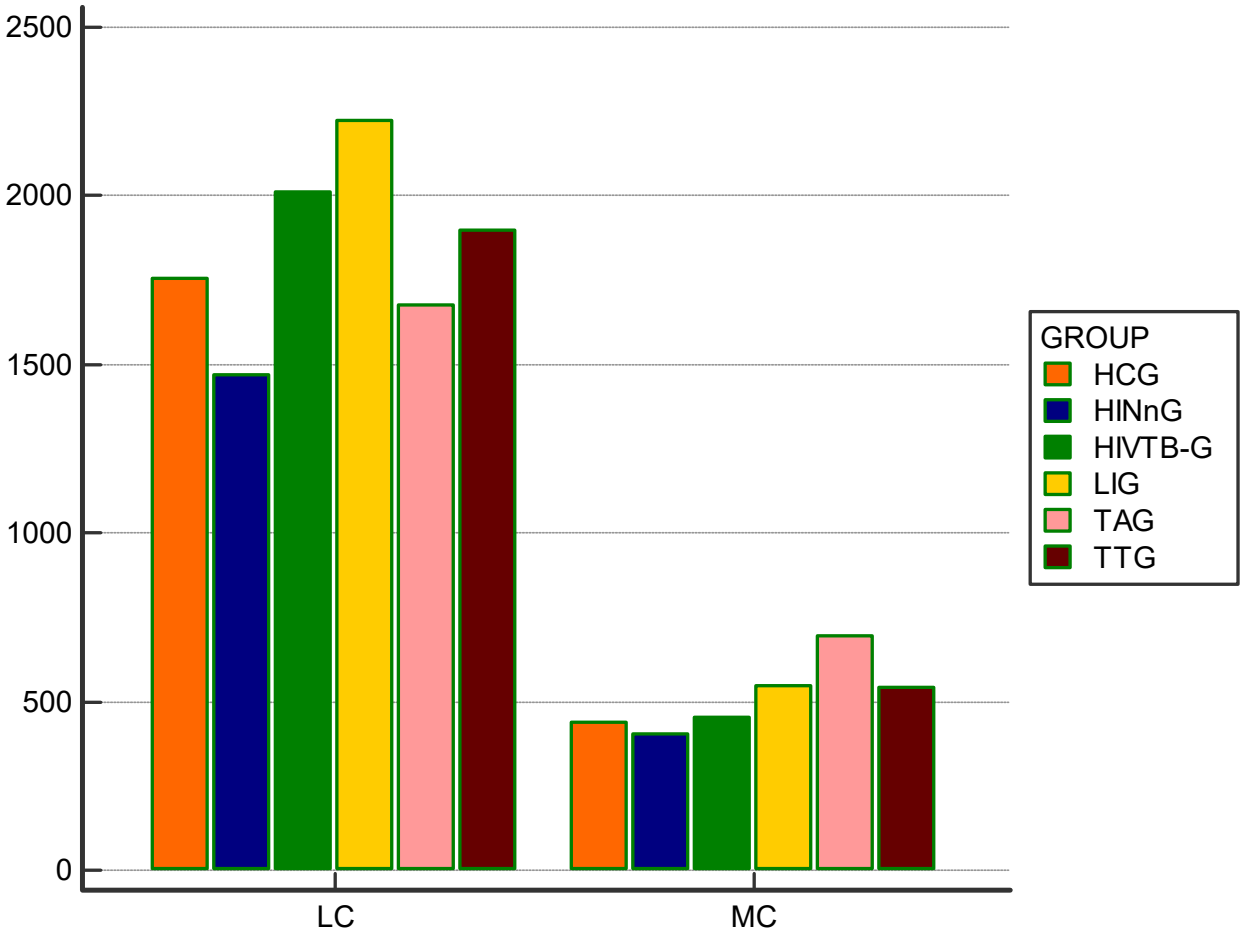

Figure 1. The mean value of lymphocyte absolute blood cell count (LC) and monocyte absolute blood cell count (MC) for every group

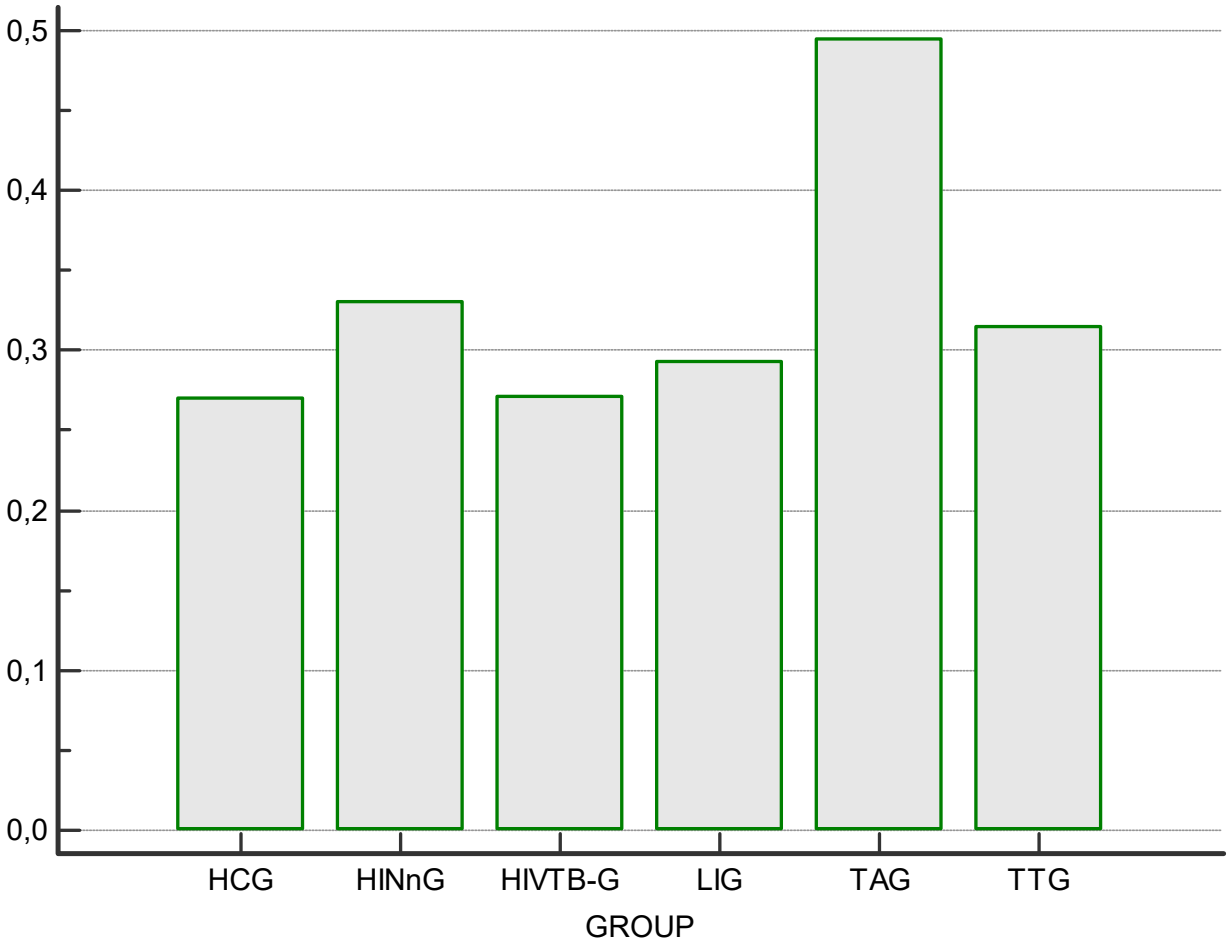

Figure 2. Mean score of monocytes / lymphocytes for every group