

Assessment of immuno-hematological effects due to helminthiasis in patients with *Mycobacterium tuberculosis* complex infection in Bobo-Dioulasso, Burkina Faso

Diakourga Arthur Djibougou^{1,2,3}, Gloria Ivy Mensah⁴, Achille Kabore⁵, Leon Tinnoga Sawadogo^{1,6}, Lompo Felix⁷, Amariane Kone², Hervé Mwinone Hien^{2,3}, Bassirou Bonfoh⁸, Kennedy Kwasi Addo⁴, Adrien Marie-Gaston Belem¹, Clement Ziemlé Meda^{1,2}, Roch Konbohr Dabiré^{2,3}, Jonathan Hoffmann⁹, Matthieu Perreau¹⁰, Potiandi Serge Diabougou^{3,7}

¹Université Nazi BONI, Bobo-Dioulasso, Burkina Faso; ²Centre MURAZ/Institut National de Santé Publique, Bobo-Dioulasso, Burkina Faso; ³Institut de Recherche en Sciences de la Santé/CNRST, Ouagadougou, Burkina Faso; ⁴Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana; ⁵FHI 360, Infectious Disease and Health Systems (IDHS), Washington DC, USA; ⁶Programme National Tuberculose, Ministry of Health, Ouagadougou, Burkina Faso; ⁷Etudes Formation et Recherches Développement en Santé (EFORDS); ⁸Centre Suisse de Recherches Scientifique de Côte d'Ivoire, Abidjan, Côte d'Ivoire; ⁹Département Médical et Scientifique, Fondation Mérieux, Lyon, France; ¹⁰Faculty of Biology and Medicine, Université de Lausanne, Lausanne, Switzerland

BACKGROUND

- Approximately 2 billion people are affected by helminthiasis, and it is one of the major burdens of developing countries. Similarly, tuberculosis (TB) affects more than one-third of the world's population and remains a major public health problem (Amin et al., 2023; WHO, 2022).
- The geographical distributions of helminth infections and TB overlap considerably, especially in developing countries, which increases the risk of coinfection with both diseases.
- In case of coinfection, helminths induce immunomodulatory reactions through a humoral T helper-2 (Th2) immune and profound hematological abnormalities (Makepeace et al., 2012; Resende et al., 2007).

- In Burkina Faso, the prevalence of intestinal parasitic infections at the reference hospital in Bobo-Dioulasso was 65.3% in 2015 and the incidence of TB is estimated at 45/100,000 inhabitants in 2021 (Sangare et al., 2015; WHO, 2022). However, there is no data on the outcomes of TB and helminth coinfection in humans.
- We analyzed the influence of helminths on immuno-hematological parameters in patients with newly diagnosed active pulmonary tuberculosis with or without concomitant helminthiasis in the city of Bobo-Dioulasso in western Burkina Faso.

METHODS

- Type and study setting:** Prospective cross-sectional Study conducted in Bobo-Dioulasso from March 2nd, 2019 to July 30, 2021, from Regional TB Control Centre of Bobo-Dioulasso (CRLAT), the Medical Center of Dô (CMA de Dô) and the Medical Center of Dafra (CMA de Dafra) in Bobo-Dioulasso (11°10'42" N; 4°17'35" W) (Fig. 1).

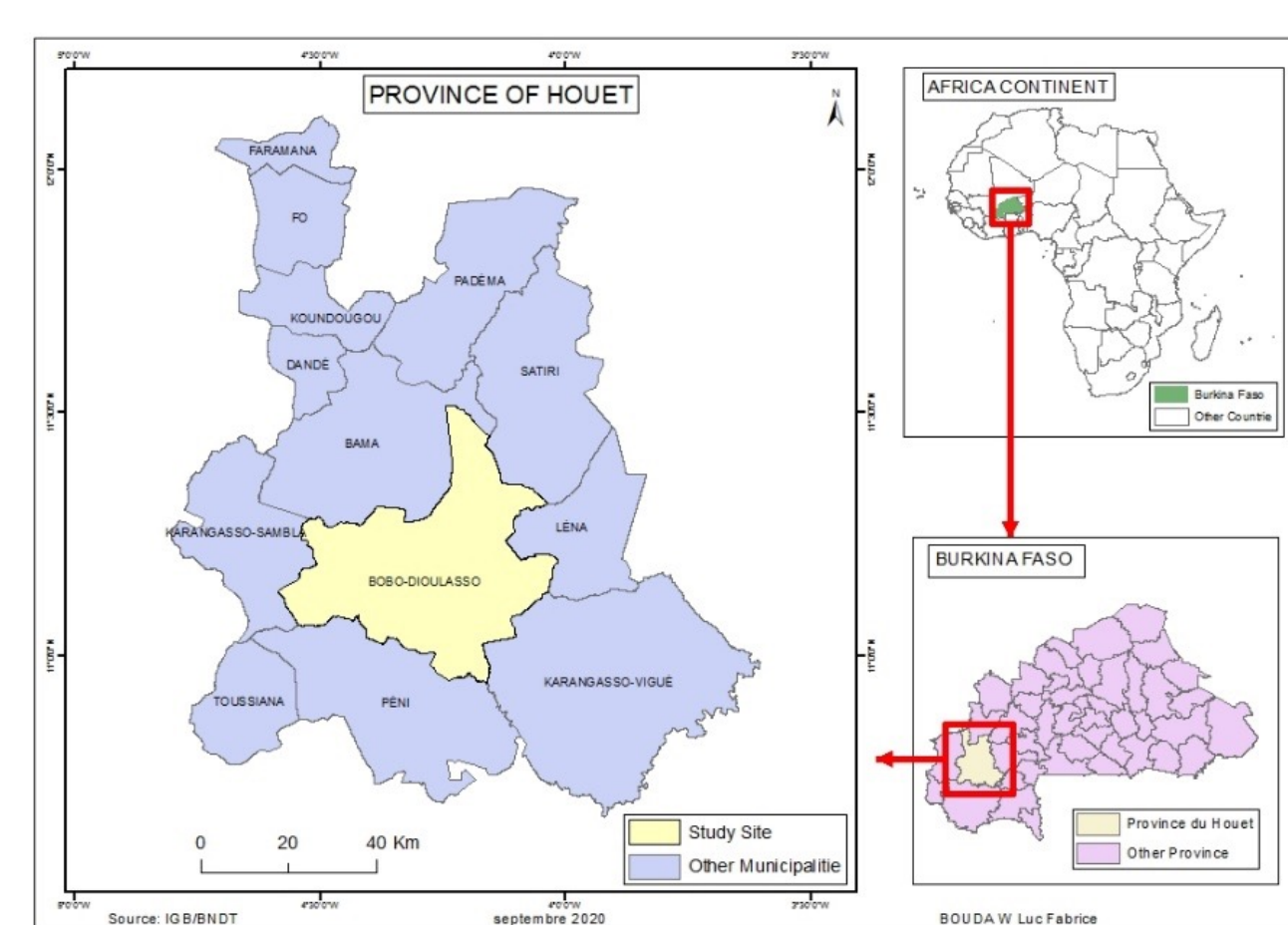
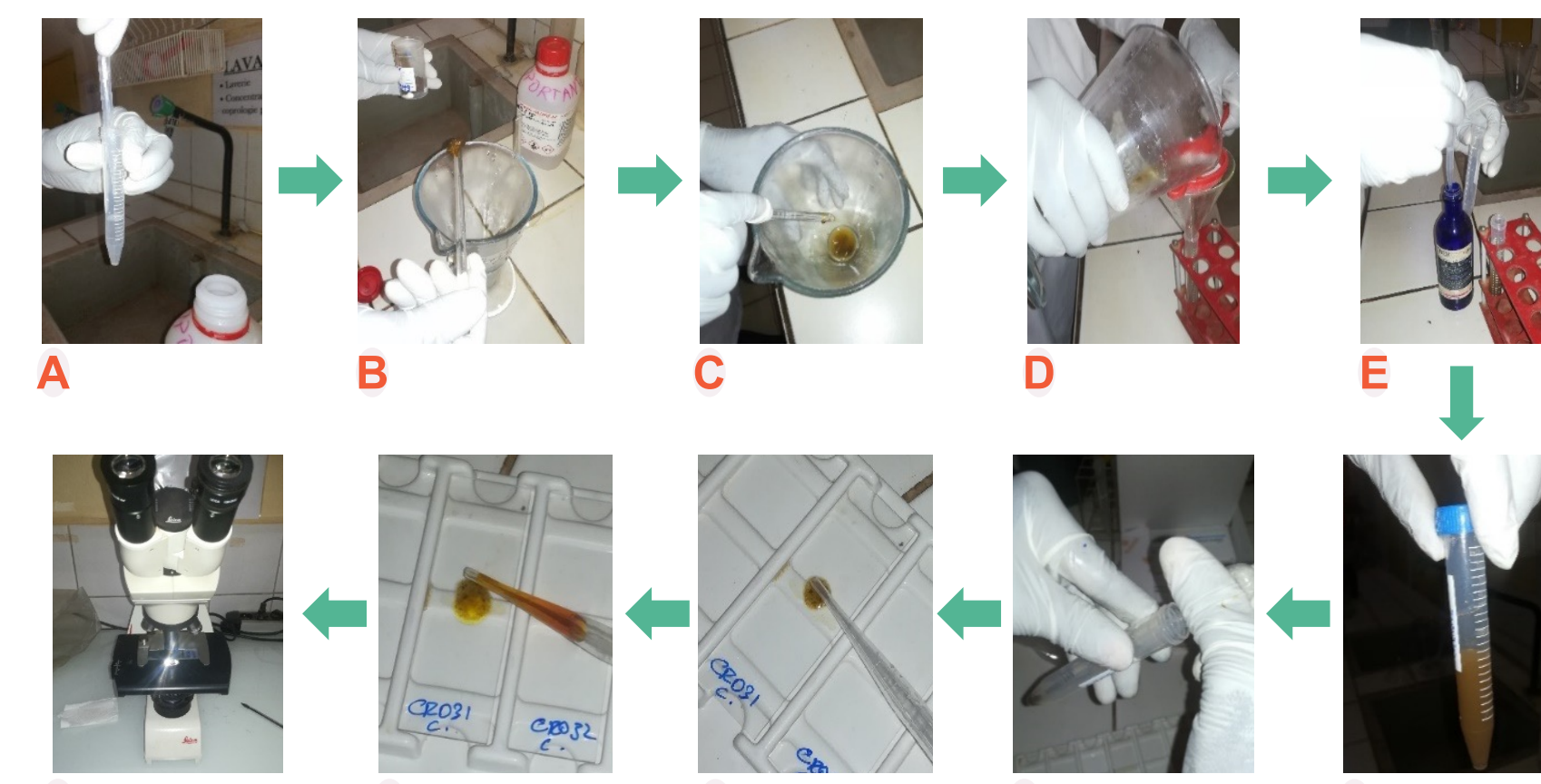


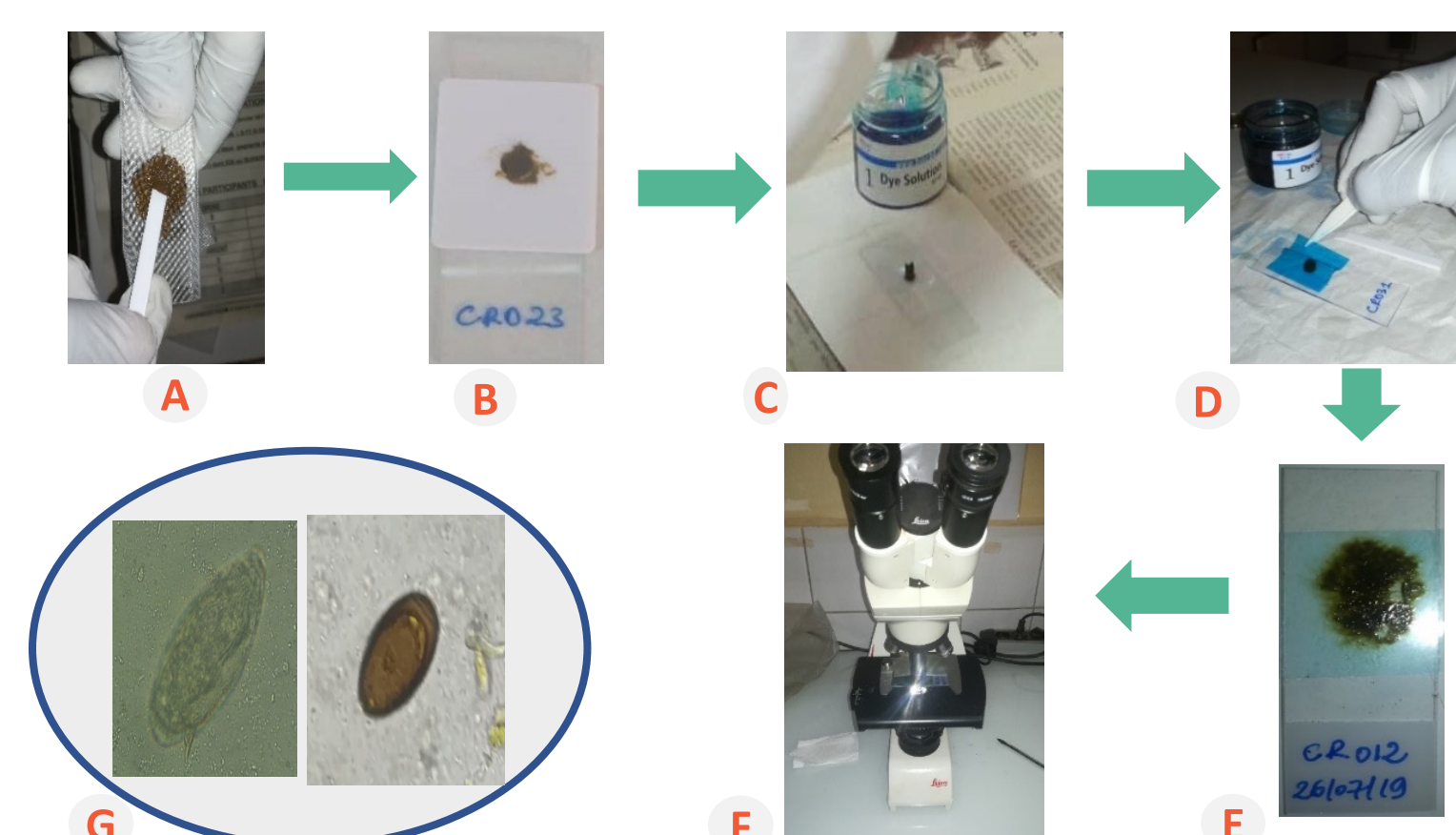
Figure 1: Study area

- Populations studied:** Newly diagnosed TB patients and Non-TB group. Participants divided into three subgroups: group1 (n=82) as control were participants without helminth nor TB infection (Mtb-/ Helm-), group 2 (n=73) were TB patients without helminth infection (Mtb+/ Helm-), and group 3 (n=22) were TB patients with helminth infection (Mtb+/ Helm+)
- Sample collection and Laboratory procedures:** A stool sample for parasitological analysis (FEC and Kato-Katz (Fig. 2 and Fig. 3)), and a 4 mL blood sample in an ethylenediaminetetraacetic acid tube (for immuno-hematological tests (Fig. 4 and Fig.5)) were collected from each participant.



A to C- Salted formalin is added to the stool. D to F- Filtration of the mixture and addition of ether. G to I- Spreading the pellet between slide and coverslip after concentration.

Figure 2: Formol-ether technique



A- Stool sieving on a calibrated plate. B- Obtaining a given quantity of stool. C to E- Cover the preparation with a cellophane slide impregnated with lightening solution. F- Examine the spread under the light microscope at the x10 objective and then at the x40 objective. G- Illustration of *S. mansoni* and *D. dendriticum* eggs under the microscope.

Figure 3: Kato-Katz technique



Figure 4: Hematological analyses using Symex XN-551 Machine



Figure 5: Fastcount BD for immunological biomarkers assessment

- Data analyses** Using R software version 4.1.0.

RESULTS

1. Characteristic of study populations

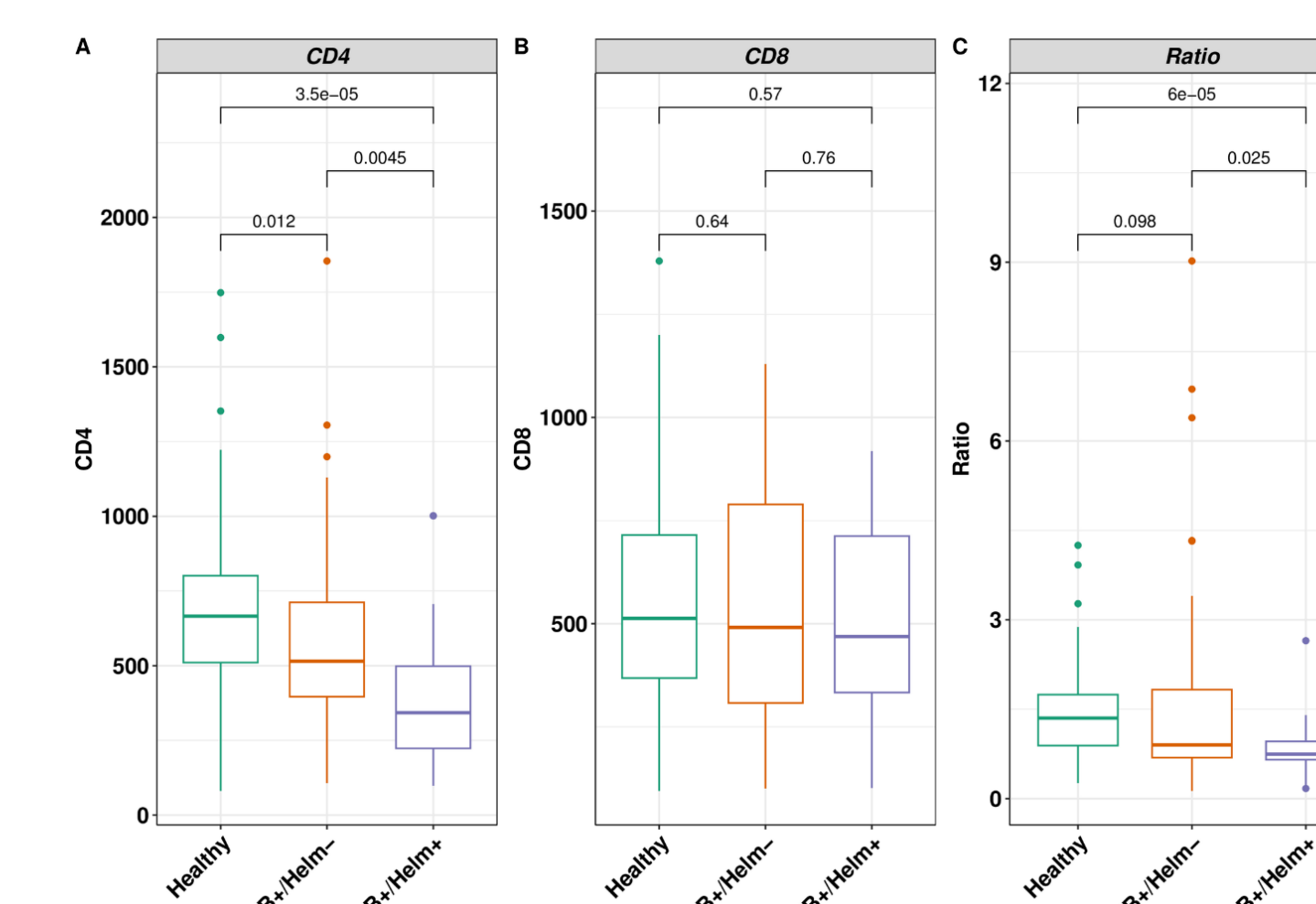
The median age 37 (iqr=17), 34 (iqr=17), 34.5 (iqr=19) for Mtb- / Helm-, Mtb+ / Helm-, Mtb+ / Helm+ respectively.

2. Profile of helminths encountered in the study population

Helminth species	TB patients coinfectd n= 22	Frequency % (22/95)
<i>Schistosoma mansoni</i>	17	77.27
<i>Dicrocoelium dendriticum</i>	3	13.63
<i>Enterobius vermicularis</i>	2	9.09
<i>Wuchereria bancrofti</i>	1	4.54
<i>Hymenolopis nana</i>	1	4.54

3. Relationship between lymphocyte subpopulations (CD4, CD8), CD4:CD8 ratio and helminths infection status (Fig. 6)

A low CD4 T cell count and a low CD4:CD8 ratio were significantly associated with TB+/Helm+ coinfection compared to the other groups (p<0.05) (Fig. 6).

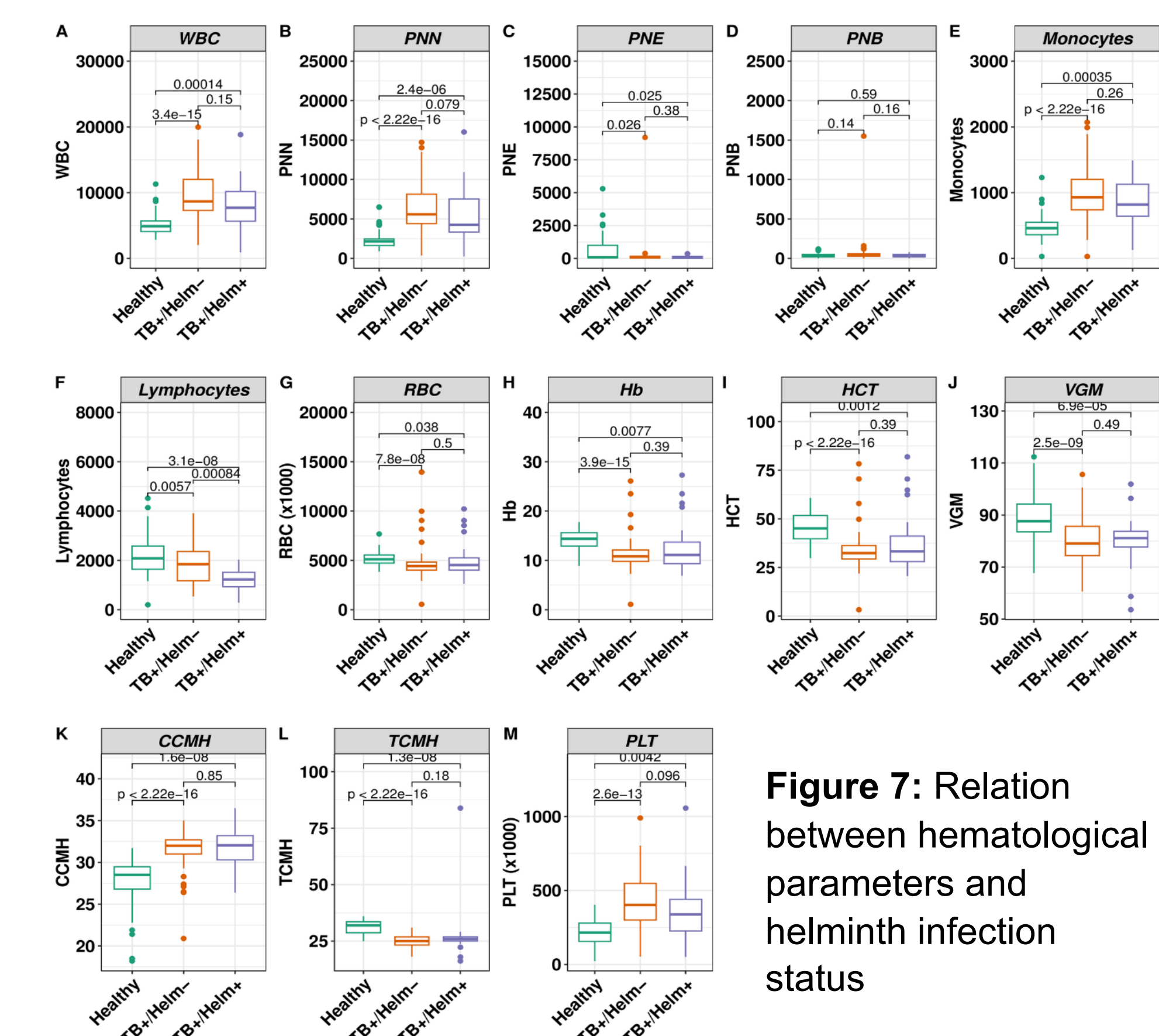


A to C- Mean of CD4, CD8 T cells subpopulations and CD4:CD8 ratio in blood from healthy control participants (TB-/Helm- or Mtb-/Helm-), TB patients without helminths (TB+/Helm- or Mtb+/Helm-) and TB patients with helminth infection (TB + Helm or Mtb+/Helm+), patients. Results are expressed as mean or median. Significant differences between groups are depicted in the figure with their respective p-values and were calculated using appropriate test.

Figure 6: Relationship between immunological parameters and status of infection

4. Relation between hematological parameters and helminth infection status

Lymphopenia was associated with concomitant helminth infection (p<0.05) (Fig. 7).



Mean values of the white blood cells (WBC) (A), Mean values of the blood components platelets (M), Mean of Red blood Cells (RBC) (G), Mean of WBC subsets such as neutrophils (B), eosinophils (D), basophils (E), lymphocytes (F), monocytes (E), and means of hematimetric constants such as hemoglobin rate (H), hematocrit rate (I), VGM/MCV (J), Mean corpuscular hemoglobin concentration (MCHC/CCMH) (K), MCH/TCMH (L) and mean of platelets (PLT) (M). Significant differences between groups are depicted in the figure with their respective p-values and were calculated using appropriate test.

Figure 7: Relation between hematological parameters and helminth infection status

CONCLUSION

- Concomitant infection with helminths and *Mtb* complex was associated with significantly low CD4 T cells count and a low CD4/CD8 ratio.
- Of the various hematological abnormalities observed in TB patients, only lymphopenia was associated with concomitant helminth infection.
- Exploring these types of immuno-hematological biomarkers would constitute an additional lead in the diagnosis and a better follow-up and monitoring of the TB-helminthiasis coinfection.

