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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF IMPORTED MALARIA IN ADULTS IN MILAN, ITALY, 2010-2015

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Malaria is the most common and lethal parasitic disease worldwide with an estimated 216 million of cases and 445,000 deaths in 2016 [1]. In Europe, where the disease has been eradicated malaria was responsible of over 8000 imported cases in 2015, two-thirds of which reported in France, the United Kingdom, Italy, Spain and Germany. The aim of this retrospective study was to describe the epidemiological and clinical characteristics of the imported malaria cases observed in Milan in the period 2010-2015. In addition, we compared it with those described in other recently published European studies [2-10]..

During the study period, 180 cases of malaria were diagnosed in 177 patients (124 males, 70.1%, and 53 females, 29.9%). Their median age was 38 years (IQR 29-49); 117 (66%) were aged <45 years. Sixty-three (35.6%) were Italian citizens, seven European citizens, and 107 (60.5%) extra-European citizens (all originating from Africa except for five patients from Pakistan, two each from India and Brazil, and one from Thailand). The African patients (97, 54.8%) originated from eighteen countries, with Eritrea (20, 20.6%), Senegal (18, 18.6%), Nigeria (12, 12.4%) and the Ivory Coast (9, 9.3%) being the most represented.

The average annual number of cases diagnosed at our hospital during the study period was 30, with the highest number of cases (40) in 2015 and the lowest in 2011 and 2012 (22). Cases were diagnosed in every month of the year, with peaks in September and October (32 cases each, 35.5%). Fifty-seven patients (32%) were classified as VFRs; 34 (19%) as immigrants, 51 (28.8%) as people travelling for working reasons and 20 (11%) for tourism; fifteen (9%) were expatriates, missionaries or people working in non-governmental organisations.

The median duration of travel for tourism was 14 days, which was significantly shorter than the 33 day spent in malaria-endemic areas by VFRs (p=0.001). Malaria was caused by *P. falciparum* alone in 136 cases (75.5%), *P. vivax* alone in 30 cases (16.7%), *P. ovale* alone in seven cases (3.9%), and *P. malariae* alone in four cases (2.2%). There were three cases of mixed infections (1.7%) caused by *P. falciparum* + *P. vivax*. All but one of the episodes of malaria due to *P. falciparum* alone were

acquired in Africa with West Africa (98, 72.1%) being the most frequent travel destination or place of origin. The distribution of the areas in which *P. vivax* malaria was acquired was more widespread, with the highest number occurring in Eritrea (16, 53.3%) and the Indian subcontinent (7, 23.3%). All of the cases of *P. ovale* and *P. malariae* were acquired in Africa .The median time from presentation at the Emergency Department (ED) of our hospital and parasitological diagnosis of malaria was three hours (IQR 2-5 hours), and the median time between the parasitological diagnosis and the start of antimalarial therapy was also three hours (IQR 1-4.1 hours).

Severe *P. falciparum* malaria (in one case associated with *P. vivax*) requiring Intensive Care Unit (ICU) admission was diagnosed in 12 patients (6.6%); five more patients (2.8%) managed outside the ICU fulfilled the criteria for severe malaria: 2 hyperparasitaemia, 2 jaundice (with parasitaemia > 100,000/uL) and one hypotension. All of the other episodes were classified as uncomplicated malaria. The most common symptoms at the time of diagnosis were fever (174, 96.6%), headache (87, 49%), arthralgia/myalgia (74, 41.1%) and chills (71, 40%); gastrointestinal symptoms were less frequent: nausea in 36 cases (20%), vomiting in 42 cases (23%), diarrhea in 32 cases (18%), and abdominal pain in 19 cases (10.5%).

The use of chemoprophylaxis was recorded for only 18 patients (10%), but was considered appropriate in 14 cases (73.7%). Thrombocytopenia was the most frequent hematologic alteration (observed in 79% of the cases), with a median value of 93,500/μL (IQR 54,000-142,750/μL). There was no difference in the prevalence of thrombocytopenia between the patients with *P. falciparum* malaria and those with *P. vivax* malaria (78.6% *vs* 73.3%). Anemia was detected in 48 cases (26.6%), but was statistically significantly more frequent among the patients with *P. vivax* malaria than among those with *P. falciparum* malaria (53.3% *vs* 19.1%, $p<0.0001$). Severe anemia (Hb ≤7 g/dL) was detected upon admission in only three patients: two with *P. falciparum* malaria and one with *P. vivax* malaria. Forty-nine patients (27.4%) presented leukopenia, with a median value of 5080/μL (IQR 3835-6770/μL).

The most frequent laboratory alterations were high serum lactate dehydrogenase levels (> 220 U/L) (112 cases, 62.2%) and increased bilirubin levels in 94 cases (52.5%) of whom 24 cases (13.3%) had levels above the 3 mg/dl. The median parasitaemia value in the case of *P. falciparum* malaria was 42,900/μL (IQR 9,058-106,750), with 26 patients (20.5%) having a parasite count of >100,000/μL (excluding 10 patients with severe malaria admitted to the ICU the remaining cases involved semi-immune African patients). The median *P. vivax* parasitaemia value was 8,290/μL (IQR 1,867.25-45,750), with one patient having a parasite count of >100,000/μL.

One hundred and two blood samples (56.6%) taken at the time of diagnosis were concurrently tested with the immunochromatographic test (RDT, Core malaria Pan/PV/PF, CORE Diagnostics, Birmingham, UK) with the following positive results: *P. falciparum* 75/75 (100%), *P. vivax* 15/20 (75%), *P. ovale* 0/5 (0%) and *P. malariae* 0/2 (0%), for an overall sensitivity of 88.2%.

All of the patients were hospitalised regardless of the infecting *Plasmodium* species, and treatment was received in 179 cases (99.4%: one patient with *P. vivax* malaria abandoned the hospital before starting any treatment).

The patients with *P. falciparum* malaria were most frequently treated with mefloquine (34.1%) and dihydroartemisinin-piperaquine (30.4%), which became the drug of choice for the treatment of uncomplicated *P. falciparum* malaria in our internal guidelines when it was registered in Italy in 2013; quinine (alone or in combination with doxycycline) was used in 44 cases (24.6%).

Chloroquine was most frequently used to treat non-*falciparum* malaria (80.5%).

Parasite clearance was faster in the patients with uncomplicated *P. falciparum* malaria treated with dihydroartemisinin-piperaquine (a median of 43.75 hours) than in those treated with mefloquine (63 hours) or quinine (72 hours) ($p < 0.0001$), but there was no statistically significant difference in defervescence time.

The median duration of hospitalisation was shorter in the case of the patients treated with mefloquine (4 days, IQR 3-5 days) or dihydroartemisinin-piperaquine (4 days, IQR 3-5 days) than

among those treated with quinine (7 days, IQR 4-7 days; p<0.01). Outcomes were favourable in all cases, and relapses occurred in only two patients (one with *P. vivax* and the other with *P. ovale* malaria), neither of whom was treated with primaquine.

VFRs are still the most affected risk group (32%), although the percentage is less than that reported in Spain and the UK [3-5] but also lower than that observed in other studies most of which performed in Italy [8-10] (Table 1). Nineteen percent of our cases involved recent immigrants, most of whom were young refugees from Eritrea infected with *P. vivax*.

We are not aware of any study of imported malaria that has recorded the time between a patient's arrival at an Emergency Department (ED) and the subsequent parasitologic diagnosis of malaria or the time between diagnosis and the start of antimalarial treatment, although these are possible indicators of good and efficient clinical practice. In our case, these times were both very short (a median of 3 hours), possibly because our ED staff always includes a specialist in infectious diseases, an organisational condition that, to the best of our knowledge, does not exist at other Italian hospitals. The frequency of severe falciparum malaria varies widely in the European studies cited above (from 4.6% in Spain to 36.3% in UK) possibly because of the different criteria for severe malaria adopted [3-10]. The determination of HIV serology is recommended in febrile tropical travellers, but the findings of three recent studies from Spain and the UK indicate that compliance with this recommendation is only 27-48% [3-5]. We established the HIV serostatus of 83% of our patients (147/177) at the time of the diagnosis of malaria, and found that 1.4% were HIV positive, which is less than the 8.9% prevalence observed in Fuenlabrada, Spain [4]. Given the large number of patients diagnosed with malaria originating from sub-Saharan Africa, and the possible negative effect of HIV on malaria outcomes, HIV serology should be offered to all patients and test monitoring should be considered an indirect index of the quality of care.

The immunochromatographic test used in our study had an overall sensitivity of 88.2% and, as expected, only performed satisfactorily in the case of *P. falciparum* malaria thus confirming that

an RDT should always be coupled with a blood smear examination in settings in which malaria is not endemic.

In comparison with our previous study [2], there was a considerable decrease in the use of mefloquine (from 71.9% to 34.1%) to treat uncomplicated falciparum malaria, and a parallel increase in the use of dihydroartemisinin-piperaquine (DHA-PPQ), the only artemisinin combination therapy (ACT) registered in Italy. We found that parasite clearance was faster in the patients receiving ACT than in those receiving mefloquine or quinine, and the duration of hospitalisation was shorter than in the case of those treated with quinine. In conclusion, *P. falciparum* remains the main species responsible for imported malaria in Milan, Italy, with VFRs and first arrival migrants being at the highest risk. *P. vivax* malaria was diagnosed in the majority of cases in asylum seekers from Eritrea. The use of chemoprophylaxis is still negligible among subjects at higher risk of developing malaria. Early and accurate diagnosis with prompt treatment of clinical malaria are essential for reducing malaria morbidity and mortality.

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Ethical approval of the study protocol was obtained from the *Comitato Etico Milano Area 1* (Study Registry No. 2016/ST/264) on 5 July 2017 (Protocol No.18186/2017). The data were anonymised before the statistical analysis.

Conflicts of interest

None of the authors has any conflict of interest requiring disclosure.

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Table 1- Summary of recent studies regarding imported malaria in Europe

Author, reference	Rey, 3	Antinori, 2	Stepien, 6	Fernandez Lopez, 4	Francis, 5	Modol, 7	Luise, 8	Zanotti, 9	Calderaro, 10
City, Country	Madrid, Spain	Milan, Italy	Poland	Madrid, Spain	London, United Kingdom	Barcelona, Spain	Padua, Italy	Brescia,Italy	Parma,Italy
Period of study	2005-2008	1998-2007	2003-2011	2004-2014	2013-2015	2007-2016	2005-2015	1999-2016	2013-2017
Type of the study	Retrospective, single center	Retrospective, single center	National surveillance	Retrospective, single center	Retrospective, single center	Prospective observational, multicenter	Retrospective, single center	Retrospective, single center	Prospective observational , single center
N° patients	57	291	189	185	133	190	124§	975§	69§
Male sex (%)	24 (42.1%)	186 (64%)	139 (74%)	Not reported	86 (64.7%)	121 (63.7%)	90 (72.6%)	742 (76.1%)	66 (74.2%)
Age (years), median	27.8*	35	36	30.8*	41	32*	39	37.8*	Not reported
Species of *Plasmodium* - *P. falciparum* - *P. vivax* - *P. ovale* - *P. malariae* - Coinfections - Unknown	 54 (94.7%) - 3 (5.3%) - - -	 228 (78.3%) 48 (16.5%) 9 (3.1%) 1 (0.3%) 5 (1.7%) -	 115 (60.8%) 35 (18.5%) 3 (1.6%) 3 (1.6%) 6 (3.2%) 27 (14.3%)	 167 (90.3%) 3 (1.6%) 3 (1.6%) 3 (1.1%) 6 (3.2%) -	 102 (76.7%) 23 (17.3%) 3 (2.3%) 2 (1.5%) 3 (2.3%) -	 122 (64.2%) 44 (23.2%) 2 (1.1%) 3 (1.6%) 5 (2.6%) 14 (7.4%)	 110 (88.8%) 6 (4.8%) 0 (0%) 3 (2.4%) 2 (1.6%) 3 (2.4%)	 820 (84.1%) 96 (9.8%) 37 (3.8%) 20 (2.1%) 2 (0.1%) -	 58 (84%) 2 (2.%) 8 (11.6%) 1 (1.4%) 2 (2.9%)
Chemoprophylaxis - Correct	6/55 (10.9%) 1/6 (16.7%)	61/258 (23.6%) 32/61 (52.5%)	62/189 (33%) 6/62 (9.7%)	29/185 (15.7%) 8/29 (27.6%)	20/79 (25.3%) 5/8 (62.5%)	19/190 (10%) 2/19 (10.5%)	16/104 (15.4%) 3/16 (18.7%)	9/644 (1.4% 9/644 (1.4%)	Not reported
Travel reason - Tourism - VFR - Recent immigrant - Missionary - Business/work - Unknown	 2 (3.6%) 34 (61.8%) 19 (34.5%) - -	 146 (50.2%) 95 (32.6%) 35 (12%) 5 (1.7%) 10 (3.4%)	 71 (38%) 13 (7%)^ - 66 (35%) 17 (9%)	 - 159 (85.9%) 25 (13.5%) - - -	Not reported	 8 (4.2%) 171 (90%) - - 11 (5.8%) -	 15 (12.1%) 86 (69.4%) 8 (6.4%) - 15 (12.1%) - -	 40 (4.1%) 619 (63.5%) 97 (9.9%) 52 (5.4%) 41 (4.2%) - -	Not reported
HIV serology done Positive	20 (35.1%) 2/20 (10%)	Not reported	Not reported	89/185 (48.1%) 8/89 (8.9%)	36/133 (27.1%) 3/36 (8.3%)	Not reported	Not done	Not done	Not reported
Positive immunochromatography	42/57 (73.7%)	Not done	Not reported	11/11 (100%)	52/53 (98.1%)	109/124 (87.9%)	Not done	Not reported	65/69 (94.2%)
Hospital admission	9 (15.8%)	291 (100%)	Not reported	176 (95.1%)	107 (91.5%)	127 (66.8%)	124 (100%)	Not stated	Not stated
Severe *P. falciparum*	0	35/233 (15%)	38/118	8/173 (4.6%)	37/102 (36.3%)	34/127	5 (4%)	49/820 (5.9%)	Not reported

malaria			(32.2%)			(26.8%)			
Mortality	0	0	5/189 (2.6%)	0	0	0	0	2 (0.2%)	0

* mean age; ^ Not stated if VFR or recent immigrants; § In all these studies were enclosed also children that are excluded from this analysis