Helminth-related Eosinophilia in African Immigrants, Gran Canaria

Javier Pardo,*†1 Cristina Carranza,†1 Antonio Muro,* Alfonso Angel-Moreno,§ Antonio-Manuel Martín,‡§ Teresa Martín,* Michele Hernández-Cabrera,†¶ and José-Luis Pérez-Arellano‡¶

Of 788 recent African adult immigrants to Las Palmas de Gran Canaria, 213 (27.0%) had eosinophilia. The most frequent causes were filariasis (29.4%), schistosomiasis (17.2%), and hookworm infection (16.8%). Stool microscopy and filarial and schistosomal serologic tests gave the highest diagnostic yield. Country of origin and eosinophil count were associated with specific diagnoses.

We prospectively evaluated the prevalence and causes of eosinophilia in recent adult immigrants from Africa; the diagnostic usefulness of parasitologic and serologic tests; and the relationship between specific helminthic infections, country of origin, and degree of eosinophilia. After they gave written consent, 788 African immigrants were screened by examination of detailed medical records, physical examination, routine laboratory tests, serologic tests, the Mantoux test, and chest radiographs. Of the immigrants, 213 met the following inclusion criteria: 1) arrival within 6 months; 2) age ≥18 years, and 3) eosinophilia (≥0.45×10⁹ eosinophils/L). Direct parasitologic tests included the examination of 3 stool samples (both Kato-Katz and Ritchie techniques were used for each sample) and specific tests for Strongyloides stercoralis (Baermann test and agar culture) (1), optic microscopy of a terminal urine specimen, and Knott test for microfilaremia. The immune chromatographic test for Wuchereria bancrofti (ICT Filariasis Binax, Portland, ME, USA), skin snips, and the Mazotti test were also used in selected cases.

ELISAs with crude extracts of adult Dirofilaria immitis adult worm antigens (AWA Di) (2), Schistosoma bovis worm antigens (3), Fasciola hepatica excretory/secretory antigens (4), and Trichinella spiralis L1 antigens (5) were used. Polystyrene microtiter plates were coated with 100 µL antigens per well in carbonate buffer (pH 9.6). Serum diluted 1:100 was added and incubated for 1 h at 37°C. Horseradish peroxidase goat anti-human immunoglobulin G (Sigma, Saint Louis, MO, USA) was added at different dilutions. Washes were performed 3 times with 200 µL phosphate-buffered saline–Tween 20 per well. After incubation for 1 h at 37°C, the substrate solution ( ortho-phenylenediamine-H₂O₂) was added, and the reaction was stopped with 3N H₂SO₄.

Assay sensitivities were evaluated by using serum specimens from patients with a definite diagnosis of isolated helminthic disease (Table 1). In all patients, adequate parasitologic tests showed no other helminthic infection. To evaluate specificities, we used serum samples from Spanish blood donors; samples from healthy controls from sub-Saharan Africa; and samples from patients with isolated helminthic, protozoal, bacterial, or viral infections (Table 1). Healthy controls from sub-Saharan Africa were clinically evaluated; they did not have eosinophilia, and results of a systematic investigation for helminthic infections (using stool samples, urine samples, and Knotts test) were negative.

Moreover, an ELISA was used to test for strongyloidiasis with somatic larvae antigens from Strongyloides venezuelensis. Although the ELISA is 100% sensitive, its low specificity precluded its use as a diagnostic tool.

The SPSS 11.5 statistical package (available from http://www.spss.com) was used for analyses. The level of significance accepted was <0.05, and results were expressed as means plus standard deviation (SD). The receiver-operating-characteristic curve was used to establish ELISA cut-offs. The χ² and the Fisher exact tests were used to evaluate the association between demographic variables and final diagnoses, and the Student t test was used to compare the degree of eosinophilia among patients with single and multiple infections. Analysis of variance and post-hoc tests were used to compare the mean eosinophil counts in each final diagnosis.

We found that 213 (27.0%) of 788 immigrants whose conditions were analyzed had eosinophilia. Of these, 191 (89.7%) were male, with a mean age of 27.4 years (SD 8.3). Two hundred two (94.9%) patients were from sub-Saharan countries, mainly Nigeria (24.1%), Sierra Leone (17.3%), Ghana (15.0%), and Mali (8.9%); 165 (77.1%) patients had 0.450–0.999×10⁹ eosinophils/L, 47 (21.9%) had 1.000–2.999×10⁹ eosinophils/L, and 1 patient had >3.000×10⁹ eosinophils/L.

One hundred fifty-four study participants (72.3%) were asymptomatic. In symptomatic patients (28.0%), the most frequent clinical features were lymphadenopathy (6.1%), pruritus (5.6%), and skin lesions (3.3%).

*These authors contributed equally to this article.
A final diagnosis was made in 161 cases (75.6%): 116 (54.5%) had 1 parasite, 30 (14.1%) had 2, and 15 (7.0%) had >3. The most frequent parasites were filariae (n = 63, 29.6%), schistosomes (n = 37, 17.4%), hookworms (n = 36, 16.8%), and *Trichuris* spp. (n = 18, 8.4%) (Figure 1). Direct methods were used in 60 (37.2%) patients, indirect methods were used in 80 (49.6%), and both methods in 21 (13.0%) patients. Stool microscopy and filarial and schistosomal serologic testing yielded the highest positive result rates (Table 2). The country of origin was statistically associated (p<0.05) with the final diagnosis: 77% of the patients with eosinophilia from Cameroon had filariasis, 63% of the patients from Mali had schistosomiasis, and 30.8% of the patients from Nigeria had hookworm infection.

The mean eosinophil count was significantly higher in patients with a final diagnosis than in those whose conditions were not diagnosed (871 ± 431 vs. 643 ± 179) (p<0.05), and the mean count was higher also in patients with 2 or more parasites than in patients with 1 (1,045 ± 641 vs. 827 ± 389) (p<0.05). Among patients with 1 helminthic disease, those with filariasis had higher eosinophil counts than those with schistosomiasis or geo-helminthic infection (p<0.05) (Figure 2).

Eosinophilia is frequent in travelers and expatriates from tropical areas (6–12). However, its prevalence is variable (3.1%–50%), depending on the population studied (more frequent in immimmigrants than in travelers), the areas where infection occurs (mainly sub-Saharan Africa or Southeast Asia), and the design of the study (prospective or retrospective). In this prospective work, we studied a homogeneous population of immigrants who had recently arrived from Africa, and we detected eosinophilia in 27%.

Studies of persons with imported eosinophilia have made a diagnosis that identified the etiologic agent in 15% to 64% of cases (depending on the population, the selected eosinophil count, and the methods) (6–12). Using direct and serologic methods (10,13), we detected helminthic infections in 75% of the patients. In all series, the main diagnoses are filarial, schistosomal, and geohelminthic infections. Only 27.7% of our patients had related signs or symptoms, which indicates that a proper investigation can detect many asymptomatic infections.

The sensitivities of our serologic tests were >90%, with specificities of 85%–97%. Using *D. immitis* antigens for the immunodiagnosis of tropical filariasis (14), we obtained a sensitivity of 90% for microfilaremia, with 97% specificity. The utility of adult worm antigens of *S. bovis* for serodiagnosis of schistosomiasis has been recently demonstrated (3).

Our high diagnostic yield with filarial (30%) and schistosomal (28%) serologic testing is similar to that obtained by Whetham et al. in travelers returning from West Africa (10). Among the direct methods, stool microscopy was the most sensitive (35%). However, serologic testing detected another parasitic infection (mainly filarial or schistosomal) when direct tests showed only a geohelminthic infection.

Table 1. Characteristics of immunodiagnostic tests*

<table>
<thead>
<tr>
<th>Test</th>
<th>Antigen</th>
<th>µg per well of antigens</th>
<th>Serum dilution</th>
<th>Anti-IgG peroxidase dilution</th>
<th>Sensitivity, %†</th>
<th>Specificity, %‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosoma spp.</td>
<td><em>A. S. bovis</em></td>
<td>0.05</td>
<td>1:100</td>
<td>1,200</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Filaria</td>
<td><em>A. Dirofilaria immitis</em></td>
<td>0.08</td>
<td>1:100</td>
<td>1,500</td>
<td>90</td>
<td>97</td>
</tr>
<tr>
<td>Fasciola spp.</td>
<td>E/S. F. hepatica</td>
<td>0.04</td>
<td>1:100</td>
<td>1,200</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Trichinella spp.</td>
<td>L1. T. spiralis</td>
<td>0.03</td>
<td>1:100</td>
<td>1,250</td>
<td>100</td>
<td>91</td>
</tr>
</tbody>
</table>

*†IgG, immunoglobulin G; ‡AWA, adult worm antigens; E/S, excretory/secretory antigens; L1, larvae 1 antigens.

Figure 1. Final diagnosis of patients with eosinophilia. Filarial species detected by direct methods were *Mansonella perstans* (n = 13), *Loa loa* (n = 4), and *Onchocerca volvulus* (n = 1). Schistosomal species diagnosed by direct methods were *Schistosoma hematobium* (n = 10), *S. mansoni* (n = 6), and *S. intercalatum* (n = 1).

Table 2. Diagnostic yield of etiologic tests*

<table>
<thead>
<tr>
<th>Test</th>
<th>Test done, no. (%)</th>
<th>Yield of test, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool (microscopy)</td>
<td>175 (81)</td>
<td>35</td>
</tr>
<tr>
<td>Filarial serology</td>
<td>189 (92)</td>
<td>30</td>
</tr>
<tr>
<td>Schistosoma spp.</td>
<td>213 (100)</td>
<td>28</td>
</tr>
<tr>
<td>Urine (microscopy)</td>
<td>66 (30)</td>
<td>16</td>
</tr>
<tr>
<td>Knotts test</td>
<td>123 (57)</td>
<td>13</td>
</tr>
<tr>
<td>Trichinella spp.</td>
<td>208 (97)</td>
<td>11</td>
</tr>
<tr>
<td>Fasciola spp.</td>
<td>209 (97)</td>
<td>7</td>
</tr>
<tr>
<td>ICT <em>Wuchereria bancrofti</em></td>
<td>71 (33)</td>
<td>4</td>
</tr>
</tbody>
</table>

*ICT, immune chromatographic test.
This work has been supported in part by grants from the Fondo de Investigación Sanitaria (FIS reference no. 01/0685), Comunidad Canaria (PI 2003/024), and the Red de Centros de Investigación en Medicina Tropical (FIS reference nos. C03/04 and PI 05552).

Dr Pardo is a specialist in internal medicine at Hospital Clínico Universitario de Salamanca (Spain). His research interests focus on diseases imported by travelers and immigrants.

References

Acknowledgments
We thank G.H. Jenkins for his help with the English version of the manuscript.