Strongyloides stercoralis, Eosinophilia, and Type 2 Diabetes Mellitus: The Predictive Value of Eosinophilia in the Diagnosis of S stercoralis Infection in an Endemic Community

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Background. This study examines the predictive value of eosinophilia for Strongyloides stercoralis infection, as measured by enzyme-linked immunosorbent assay (ELISA) testing, in an endemic community. In remote communities, eosinophilia is frequently used as a proxy test for the presence of helmint infections. Past studies of eosinophilia and Strongyloides infection have been conducted in specific groups such as immigrants and refugees, or in subpopulations of nonendemic communities, rather than in endemic communities.

Methods. We conducted a cross-sectional study of the relationship between eosinophilia and Strongyloides ELISA serology, as part of a study into the relationship between S stercoralis infection and type 2 diabetes mellitus (T2DM) in an Indigenous community in northern Australia.

Results. Two hundred thirty-nine adults had their eosinophil count and S stercoralis ELISA serology measured in 2012 and 2013, along with other biometric and metabolic data. Eosinophilia was found to have a relatively poor sensitivity (60.9%), specificity (71.1%), positive predictive value (54.6%), and negative predictive value (76.1%) for S stercoralis ELISA positivity in this group. However, there was a more constant relationship between eosinophilia and S stercoralis ELISA positivity in patients with T2DM (negative predictive value 87.5%).

Conclusions. This study suggests that the presence or absence of eosinophilia is not an adequate proxy test for S stercoralis infection in a community where the infection is prevalent, and that the association between eosinophilia and S stercoralis ELISA positivity is more constant in patients with T2DM.

Keywords. eosinophilia; Strongyloides stercoralis; type 2 diabetes mellitus.

Infection with the soil-transmitted helminth Strongyloides stercoralis is endemic in many of the Indigenous communities of northern Australia, with prevalence of up to 41% recorded in some locations [1].

Direct microbiological tests to diagnose the infection are often impractical in these settings because multiple fresh fecal samples are required, and sensitivity is low even in ideal circumstances. The use of enzyme-linked immunosorbent assay (ELISA) serology in the diagnosis of Strongyloides infection and in conducting prevalence surveys has, in the past, been contentious. Uncertainties exist over the meaning of positive ELISA results, what level of ELISA test should be considered positive, and whether antibodies persevere after the resolution of infection. In addition, cross-reactivity with other helmint infections was considered to be a problem, particularly with earlier versions of the test. However, recent studies suggest that the use of an ELISA test to detect antibodies to the worm is both sensitive and specific enough to diagnose the infection and determine the success of treatment, and its use is now widespread in clinical practice [2–4]. However, this test is currently not available in a point-of-care format, and therefore it entails transport of specimens to central laboratories with significant delays in diagnosis and treatment for patients in remote locations.

Eosinophilia is a common, but not uniform, finding in S stercoralis infection and is thought to be more marked in earlier infections, becoming less pronounced and more variable in chronic cases [5]. Several studies have addressed the relationship between eosinophilia and Strongyloides infection in the context of patient screening, but these have been conducted in migrant...
and traveler populations, or in subpopulations of nonendemic societies [6–12], and have often not addressed the prevalence of Strongyloides in patients without eosinophilia.

Infections that are endemic to northern Australia and that are known to produce eosinophilia include Trichuris trichuria, hookworm species, Hymenolepis nana, Toxocara canis, Giardia duodenalis, and S stercoralis [13], as well as ectoparasites such as Sarcoptes scabiei, resulting in eosinophilia being a common finding in this region.

As part of a study conducted into the relationship between type 2 diabetes mellitus (T2DM) and S stercoralis infection, we examined the prevalence of eosinophilia in relation to Strongyloides infection, as measured by ELISA serology, in an Aboriginal community. Data from this study demonstrated a negative relationship between S stercoralis infection and T2DM [1], and these data have suggested that T2DM is a predictor of treatment failure in this setting [14]. Eosinophilia is thought to be central to the process by which helminth infections can affect the metabolic status of infected subjects through a process of immunomodulation [15].

This study provides, for the first time, data on the predictive value of eosinophilia in the diagnosis of S stercoralis infection in an endemic community and analyses this in the context of T2DM.

**MATERIALS AND METHODS**

The study was conducted in 3 related Indigenous communities, located within a 100 km radius in the Tanami desert region of Western Australia. Opportunistic testing for, and treatment of, S stercoralis was commenced in these communities in 2012 according to the best practice guidelines of the Australian Strongyloides working group [16].

Strongyloides stercoralis ELISA testing was ordered, often in conjunction with other routine laboratory investigations. Testing was performed by Pathwest Laboratory in Perth Western Australia, using the commercial Strongyloides IgG ELISA (DRG laboratory). The reference values, in units of absorbance, for this test were as follows: less than 0.2 – Negative; 0.2 to 0.4 – Equivocal; >0.4 – Positive. However, as was noted by the laboratory, these ranges were developed in a metropolitan population where the prevalence of S stercoralis was very low (manufacturers information). To reduce the possibility of false-negative results, a modified range was used. All values greater than or equal to 0.30 were considered positive and treated. All values <0.30 were considered equivocal and were retested after a period of 6 months, to ascertain the rate of seroconversion (and presumably therefore new infections) in this group. Only 3 seroconversions were found in this time, and the analysis for this study was performed using the results of the initial testing only.

Data were extracted including the age, sex, date of testing, S stercoralis ELISA titer hemoglobin, total eosinophil count, percentage eosinophilia, height, weight, calculated body mass index (BMI), diabetic status and HbA1C triglyceride level, high-density lipoprotein, and total cholesterol. The study population for this study was identical to that in our 2 previous studies in this community [1, 14].

Eosinophilia was defined as a total eosinophil count of 0.50 × 109/L or greater. Diabetes was defined in this group as an HbA1C reading of 6.5% or greater, or a random blood glucose of more than 11.1 mmol/L, or a fasting blood glucose of more than 7.0 mmol/L, either at the time of testing, or in the past in patients already receiving treatment for diabetes.

**Ethical Approval**

The protocol for this study was approved in principle by the Kimberley Aboriginal Health Planning Forum. All participant were 21 years of age or older. Because no investigations or treatments apart from those required for best clinical practice were being performed, and literacy levels are very low in the study population, verbal consent was considered appropriate. Verbal consent was obtained from all participants and recorded electronically. Formal ethical approval was granted by the Western Australian Aboriginal Health Ethics Committee (HREC:515).

**Statistical Analysis**

Descriptive statistics, including means, medians, percentages, and their respective 95% confidence intervals (CIs), were used to analyze the demographic and clinical characteristics of participants. The accuracy of eosinophilia (≥0.50) as a measure of S stercoralis status (E-titer ≥0.30) was evaluated using sensitivity and specificity measures. To account for the high prevalence of S stercoralis, the positive predictive value (PPV) and negative predictive value (NPV) for the ≥0.50 eosinophilia cutoff were also calculated. These diagnostic test evaluations were also undertaken separately for participants with and without diabetes.

Four logistic regression analyses were undertaken to examine the association between diabetes status and the sensitivity, specificity, PPV, and NPV of eosinophilia as measure of S stercoralis. As a first step, dichotomous variables were created to flag participants that were true-positive cases (eosinophilia ≥0.50 and E-titer ≥0.30) or true-negative cases (eosinophilia <0.50 and E-titer <0.30).

The sensitivity regression was the odds of diabetic participants with S stercoralis having a positive eosinophilia diagnosis (true positive) compared with the odds of the same true-positive diagnosis among nondiabetic participants. The specificity regression was the odds of a true negative diagnosis by these diabetes groups.

Analysis of PPV was the odds of a true-positive diagnosis among diabetic patients with a positive eosinophilia compared with nondiabetic patients. The NPV regression was the odds of true-negative diagnosis among those with a negative eosinophilia result, again by diabetes status.

All regression analyses were also adjusted for sex, age, BMI, and previous anthelmintic treatment with albendazole. Cases with missing values on any of these variables were dropped from the adjusted modeling. A 5% significance level was used.
RESULTS

Two hundred fifty-nine patients were screened, with 92 (35.5%; 95% CI, 29.9%–41.6%) cases of Strongyloides infection, as indicated by ELISA serology of ≥0.30, diagnosed. However, eosinophil counts were available for only 239 patients, with the missing values being due to specimen degradation during transport. Of the 20 degraded specimens, 5 were positive for S. stercoralis by ELISA testing and 7 had T2DM. Table 1 details the clinical characteristics of the 239 patients with both ELISA testing results and eosinophil counts.

Table 2 shows that 97 patients had eosinophilia, giving an overall prevalence of 40.6% (95% CI, 34.5%–47.0%). In the infected patients, the prevalence was 60.9% (50.1%–70.7%). The prevalence of eosinophilia was similar in diabetic compared with non-diabetic subjects (41.9% [95% CI, 33.5%–50.9%] and 39.1% [95% CI, 30.5%–48.5%], respectively; odds ratio [OR] = 1.12, 95% CI, 0.67%–1.88, P = .659), whereas the prevalence of eosinophilia was higher in infected diabetics compared with infected non-diabetics, although this difference did not reach statistical significance (71.0% [95% CI, 51.8%–84.8%] and 55.4% [95% CI, 41.9%–68.1%], respectively; OR = 1.97, 95% CI, 0.77%–5.03, P = .156).

One quarter (25.0%) of the diabetic patients had a positive S. stercoralis ELISA test compared with almost half of the non-diabetic patients (48.7%). Eosinophilia as a test for S. stercoralis had a sensitivity of 60.9% and a specificity of 71.1% (Table 3).

Table 1. Clinical Characteristics of Study Participants (n = 239)

| Variable          | N   | Mean, Median or % | 95% CI, IQR        |
|-------------------|-----|------------------|-------------------|
| Age (years)       | 239 | 43.6             | (41.8–45.5)       |
| Male              | 99  | 41.4%            | (35.8–48.4)       |
| Weight (kg)       | 235 | 81.0             | (78.2–83.8)       |
| BMI (kg/m²)       | 226 | 29.5             | (28.5–30.5)       |
| Hb (g/L)          | 237 | 133.6            | (131.4–135.7)     |
| HbA1c %           | 204 | 6.8%             | (5.9–8.9)         |
| SBP (mmHg)        | 239 | 126.8            | (124.4–129.3)     |
| DBP (mmHg)        | 239 | 79.5             | (78.0–80.9)       |
| Cholesterol (mmol/L) | 211 | 4.6              | (4.4–4.7)         |
| HDL (mmol/L)      | 211 | 0.90             | (0.87–9.9)        |
| Triglycerides (mmol/L) | 211 | 2.1a             | (1.4–3.0)         |
| Diabetes          | 124 | 51.9%            | (45.3–58.1)       |
| Eosinophil count  | 239 | 0.43%            | (0.26–0.71)       |
| Eosinophil %      | 238 | 5.55%            | (3.40–8.50)       |
| E (ELISA) titer   | 239 | 0.15%            | (0.08–0.49)       |
| % E-titer ≥0.3    | 87  | 36.4%            | (30.5–42.8)       |
| Past anthelmintic therapy | 72  | 30.1%            | (24.9–36.7)       |

Abbreviations: BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; ELISA, enzyme-linked immunosorbent assay; Hb, hemoglobin; HDL, high-density lipoprotein; IQR, interquartile range; SBP, systolic blood pressure. * Median and IQR.

Eosinophilia had an overall PPV of 54.6% and a NPV of 76.1%. The NPV of eosinophilia was higher in diabetic patients (87.5%, 95% CI, 77.6–94.1) compared with non-diabetic patients (64.3%, 95% CI, 51.9–75.4).

Because the ELISA cutoff level of 0.30 units was used in this study for purely clinical reasons, the analysis was repeated using the conventional cutoff of 0.40 units (Supplementary Tables 1–3) and again using a higher cutoff of 0.50 for the purposes of comparison. The NPV of eosinophilia remained high for both cutoff levels (81.0% for ≥0.40 and 86.6% for ≥0.50), and the differences between diabetic and non-diabetic patients remained comparable.

Logistic regression (Table 4) showed that among the 97 patients with eosinophilia, the odds of having a positive S. stercoralis ELISA test (PPV) were 67% lower among diabetic patients compared with those without diabetes (OR = 0.33, 95% CI, 0.33–0.81, P = .010). This difference remained after adjusting for age, sex, weight, BMI, and past treatment with anthelmintics (n = 97, OR = 0.30, 95% CI, 1.11–81, P = .018). In comparison, diabetics without eosinophilia were almost 4 times more likely to also have a negative S. stercoralis ELISA test (NPV), compared with non-diabetic patients without eosinophilia (n = 142, OR = 3.89, 95% CI, 1.66–9.12, P = .002). This difference remained after adjustment, although 14 patients were excluded due to missing values on potential confounding variables (n = 128, OR = 4.51, 95% CI, 1.73–11.76, P = .002).

DISCUSSION

This study suggests that eosinophilia alone cannot be used to infer the presence of Strongyloides infection in patients from Indigenous communities where the condition is endemic. This is
perhaps not a surprising finding given the large number of other parasitic infections that are present in these communities and capable of causing eosinophilia.

However, the relatively low NPV of eosinophilia was more notable, suggesting that the absence of eosinophilia does not reliably rule out the diagnosis of *Strongyloides* infection in this setting. In fact, in this study, almost one quarter of patients without eosinophilia had positive *Strongyloides* ELISA serology. This is of relevance to clinicians because, in a remote setting, the absence of eosinophilia is often used as a proxy test to imply the absence of parasitic infection. This study suggests that such a practice would not be safe as a means to exclude *Strongyloides* infection, particularly in situations in which the patient faces immunosuppression or chemotherapy. Immunosuppression and or steroid therapy in the presence of undiagnosed *Strongyloides* infection can lead to hyperinfection syndrome, often with fatal consequences [17].

The findings are similar to those of Naidu et al [7] in their survey of refugee populations in Canada, where they are also at pains to point out that the absence of eosinophilia should not be used to infer the absence of *Strongyloides* infection. However, they are at odds with a survey of farm workers carried out in southern Spain where the results suggested that eosinophilia had a specificity of 93.1% and sensitivity of 93.5%, and the authors recommended public health screening for strongyloidiasis using eosinophilia [12]. It is clear that the situation in this community is quite different, with a low level of transmission overall and the absence of other significant parasitic infections. This disparity can also be explained in part by differences in the method of diagnosis used. The Spanish study used microbiological examination of stool specimens for diagnosis, a method that is known to have a low sensitivity, and to be influenced by the worm burden and subsequent numbers of larvae shed in the feces [2]. Eosinophilia is known to be more common in early

### Table 3. Predictive Value of Eosinophilia (≥0.5) for *Strongyloides stercoralis* Status Determined by Serology (E-Titer ≥0.3)

| Parameters       | Nondiabetic (n = 115) | Diabetic (n = 124) | Total (n = 239) |
|------------------|-----------------------|--------------------|-----------------|
|                  | n (%)                 | n (%)              | n (%)           |
| True negatives   | 45 (39.1)             | 63 (50.8)          | 108 (45.2)      |
| False positives  | 14 (12.2)             | 30 (24.2)          | 44 (18.4)       |
| True positives   | 31 (27.0)             | 22 (17.7)          | 53 (22.2)       |
| False negatives  | 25 (21.7)             | 9 (7.3)            | 34 (14.2)       |
| (%) (95% CI)     |                       | (%) (95% CI)       | (%) (95% CI)    |
| Sensitivity      | 55.4 (41.5–68.7)      | 71.0 (52–85.8)     | 60.9 (49.9–71.2) |
| Specificity      | 76.3 (63.4–86.4)      | 67.7 (57.3–77.1)   | 71.1 (63.2–78.1) |
| Positive predictive value | 68.9 (53.4–81.9) | 42.3 (29.7–56.8)   | 54.6 (44.2–64.8) |
| Negative predictive value | 64.3 (51.9–75.4) | 67.5 (77.6–94.1)   | 76.1 (68.2–82.6) |

Abbreviation: CI, confidence interval.

### Table 4. Logistic Regression Analyses of Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value by Diabetes Status

| Analysis                      | Diabetes Status | Unadjusted OR | Adjusted OR* |
|-------------------------------|-----------------|---------------|--------------|
|                               | n | OR (95% CI) | n | OR (95% CI) |
| Analysis of sensitivity       |   |           |   |             |
| Nondiabetic                   | 87 | 1.00 | 82 | 1.00 |
| Diabetic                      | 31 | 1.97 (.77–5.03) | 30 | 2.07 (.73–5.84) |
| Analysis of specificity       | 152 | 1.00 | 141 | 1.00 |
| Nondiabetic                   | 59 | 1.00 | 55 | 1.00 |
| Diabetic                      | 93 | 0.65 (.31–1.37) | 86 | 0.74 (.33–1.66) |
| Analysis of positive predictive value | 97 | 1.00 | 95 | 1.00 |
| Nondiabetic                   | 45 | 1.00 | 43 | 1.00 |
| Diabetic                      | 52 | 0.33 (.14–.77)* | 52 | 0.30 (.11–.81)* |
| Analysis of negative predictive value | 142 | 1.00 | 128 | 1.00 |
| Nondiabetic                   | 70 | 1.00 | 64 | 1.00 |
| Diabetic                      | 72 | 3.89 (1.66–9.12)* | 64 | 4.51 (1.73–11.76)* |

Bold value indicates total numbers in each category.

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

* Adjusted for sex, age, BMI, and past antibiotic treatment.

*P < .05.
or acute Strongyloides infections, when worm burden and larval counts are highest, and to decline and become more variable in chronic infection, presumably through the mechanism of parasite-induced immunomodulation in the host [5, 18]. Use of fecal testing for diagnosis would therefore tend to select earlier infections, with higher worm counts and therefore higher rates of eosinophilia, whereas ELISA testing would detect more chronic infections where immunomodulation had resulted in more variable eosinophilia.

This study found that the prevalence of eosinophilia in diabetic subjects was no different to that in nondiabetic. This is at odds with the findings of a much larger cross-sectional study performed in China that demonstrated a negative relationship between eosinophilia and insulin resistance and T2DM [19]. However, the Chinese study was conducted in a population where both helminth infection and eosinophilia are less prevalent and where other, noninfectious causes of eosinophilia are likely to be more common.

Data from this community published elsewhere demonstrate a negative relationship between Strongyloides infection and T2DM [1]. It is postulated that this effect is again due to parasite-induced immunomodulation affecting the hosts’ metabolic system in chronic infections and resulting in increased insulin sensitivity. Laboratory evidence suggests that eosinophilic infiltration of adipose tissue due to helmint infection promotes the presence of alternatively activated macrophages, which in turn act to increase insulin sensitivity [15].

However, this study showed a higher prevalence of eosinophilia in ELISA-positive diabetics and demonstrates that the absence of eosinophilia in diabetic subjects is more closely linked with the absence of Strongyloides antibodies. This might be explained if the diabetic group contains a greater proportion of acute or recent infections, resulting in a more marked eosinophilia, as opposed to chronic and immune-modulated infections, where eosinophilia is less pronounced and where the past eosinophilic infiltration of adipose tissue has contributed to a lower prevalence of T2DM.

It is equally plausible that T2DM itself is responsible for the higher rate of eosinophilia. A recently published paper looked at the relationship between the human adipokine resistin and multiple helminth infections. It found that higher resistin levels were associated with a more pronounced inflammatory response, a higher worm burden, and reduced worm clearance [20]. Type 2 diabetes mellitus along with obesity and metabolic syndrome has been variably linked with elevated levels of resistin [21]. In addition, data from this study published elsewhere found that T2DM was associated with treatment failure in Strongyloides infection, suggesting that the immune response may be altered in diabetics [14].

Perceived weaknesses of this study may be that it relies on ELISA serology alone for diagnosis rather than microscopy, which is the most specific test; however, we believe this is outweighed by the superior sensitivity of the serological test. It is clear that the numbers involved in this study are small, and further larger studies may be of benefit. In addition, no distinction was made between mild, moderate, and severe eosinophilia, with the single cutoff point of \(0.5 \times 10^9/L\) being used. It may be that using a lower threshold for eosinophilia might improve the NPV of the test.

CONCLUSIONS

This study supports the use of ELISA testing for Strongyloides infection as a screening test for patients in endemic Aboriginal communities regardless of their eosinophilia status, and it suggests the practice of presuming the absence of infection in patients without eosinophilia is not a safe one. It may be that the absence of eosinophilia might be of some use in the decision to screen or treat for strongyloidiasis in the diabetic population in this community. Further studies would be needed to assess whether this is the case in other settings where the disease is endemic and to further examine the link between eosinophilia and T2DM.

Supplementary Data

Supplementary material is available online at Open Forum Infectious Diseases online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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