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QUANTITATIVE ASSESSMENT OF EOSINOPHILURIA IN *SCHISTOSOMA HAEMATOBIUM* INFECTIONS: A NEW MARKER OF INFECTION AND BLADDER MORBIDITY

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Abstract. Eosinophiluria, as quantified by measuring eosinophil cationic protein (ECP) in urinary extracts, microhematuria, egg excretion, and ultrasound-detectable bladder pathology were recorded in *Schistosoma haematobium*-infected Tanzanian school children at a baseline survey and during an 18-month post-treatment follow-up study. Significant correlations were seen between urinary ECP levels, intensity of infection, and bladder pathology. Treatment resulted in a marked reduction in prevalence and intensity of infection, in a delayed and less marked reduction in ECP levels, and in a resolution of pathology. The overall diagnostic efficiency of the ECP test (cut-off value for the ECP ≥5 ng/ml) in relation to infection was comparable with that of egg count and microhematuria, but with a better sensitivity than a single egg count. In relation to bladder pathology, the diagnostic performance of the ECP test (cut-off value for the ECP ≥25 ng/ml) exceeded that of a single egg count. In addition, the ECP was better in discriminating between different grades of bladder pathology. The present study points to the ECP as a useful marker of both *S. haematobium* infection and of associated bladder morbidity reflecting the inflammatory status of the bladder wall.

The pathologic lesions in urinary schistosomiasis due to *Schistosoma haematobium* infection result from the granulomatous host response against the schistosome eggs, whereas the adult worms cause little or no damage.[1,2] Thus, appearance of symptoms coincides with the start of oviposition. The organs primarily affected in urinary schistosomiasis are the bladder, ureter, and kidney, with the bladder being the organ most frequently affected.[2,3] The perioval inflammatory reaction against the tissue deposited eggs gives rise to pathologic changes including hyperaemia, sandy patches, granulomas, polyps, ulcers, nodules, fibrosis, and calcification of the bladder wall. Obstructive uropathy such as hydroureter and hydronephrosis may develop due to fibrosis and stenosis of the ureterale orifice.[3] Reduction or elimination of morbidity has a high priority in schistosomiasis control.[4] To reach this objective, a thorough understanding and objective criteria and measures of morbidity are necessary.

Urinary egg counts are widely used as an indirect measure of morbidity and correlate well with other measures of morbidity such as hematuria, proteinuria, and leukocyturia assessed either quantitatively or semi-quantitatively using reagent strips.[5–8] Morbidity is associated with the intensity of the infection as estimated by *S. haematobium* urinary egg counts.[9–13] However, even at similar intensities of infection, disease sequela between individuals may vary widely.[10,14] Ultrasound examination of the urinary tract has proved to be a valuable technique in direct assessment of morbidity. It is field applicable, rapid, and provides visible evidence of pathology.[15,16] However, ultrasound does not detect the early pathologic changes, i.e., the inflammatory reactions of the bladder.[17]

Eosinophils make up a high proportion of the leukocyte infiltrate of the egg granuloma in *Schistosoma* sp. infections.[18,19] In *S. haematobium* infections in humans, this is reflected by the cellular composition in the urine where eosinophils constitute a significant proportion of the leukocytes present.[20–23]

In a previous paper, we described the use of eosinophil cationic protein (ECP) measurements in the quantitative assessment of eosinophiluria in *S. haematobium* infections in Kenyan school children.[24] It was suggested that quantitative assessment of eosinophiluria using ECP could be a valuable tool in monitoring the inflammatory reactions of the urinary tract and thus a marker of morbidity. In combination with the use of ultrasonography it could allow for a dynamic evaluation of the pathologic process, i.e., resolution and reappearance of pathologic changes post-treatment, which is important for determining re-treatment schedules in control programs. The aims of the present study were to elucidate the possible use of ECP as a marker of infection and morbidity in *S. haematobium*-infected Tanzanian school children before treatment and to follow the evolution of urinary ECP in relation to infection and ultrasound-detectable pathologic changes during an 18-month follow-up study.

MATERIALS AND METHODS

Study design and study area. The study was designed as a cohort study including a baseline examination before treatment and follow-up examinations 2, 4, 6, 12, and 18 months post-treatment.[25] The baseline examination included 529 school children with a mean age of 11.5 years (range = 7–17) attending the Mikumi (n = 337, ♂:♀ = 150:187) and Msimba (n = 192, ♂:♀ = 97:95) primary schools in the Kilosa District in southeast Tanzania. This is an area of moderate to high transmission of *S. haematobium.* According to the clinical records at the Mikumi Health Center, there are very few cases of *S. mansoni* infection in the Mikumi area (Mshinda HM, unpublished data). Of the 529 children included at the baseline examination, 463, 384, 380, 432, and 422 participated in the 2, 4, 6, 12, and 18 month follow-up

examinations, respectively. A cohort of 214 children was present at all examinations.

Oral informed consent was obtained from the children and their parents or caretakers. Ethical clearance was granted by the Tanzanian Medical Research Board Coordination Committee of the National Institute for Medical Research. The study was also approved by the Danish Central Medical Ethical Committee.

Urine examination. Urine was collected between 10:00 AM and 2:00 PM. Urine filtration for egg counts (eggs/10 ml) were performed using Nucleopore (12 μm) (Costar, Cambridge, MA), membranes and a standard syringe filtration technique.[26] Dipstick analysis for microhematuria (grades 0–4) was performed using reagent strips (Hemastick; Boehringer Mannheim, Mannheim, Germany).

Urinary egg counts and dipstick analysis for microhematuria were done on 3–5 urine samples collected on 3–5 consecutive days at each survey. The highest value of the 3–5 egg counts is defined as the maximal egg count, whereas the single egg count is the egg count in the sample collected for ECP analysis.

Sampling of urine for ECP analysis. Aliquots of well-suspended urine were collected for ECP analysis. After collection, the urine samples were kept cold in a cool box during transport and were frozen at −20°C within 4 hr after collection and kept frozen until used for ECP analysis.

Extraction of urine samples for ECP analysis. One volume of well-suspended urine was mixed with 1 volume of extraction buffer (1% N-cetyl-N,N,N-trimethyl-ammonium bromide [CTAB] in 0.15 M NaCl). After 1 cycle of freeze-thawing, the sample was mixed on a vortex-mixer and centrifuged for 10 min at 3,000 × *g* at 4°C. The supernatants containing the extracted proteins were removed and used for ECP determinations.

Measurements of ECP. The ECP in urinary extracts was measured by an ELISA technique described in details elsewhere.[27] Briefly, the method is a polyclonal sandwich type ELISA using the biotin-avidin-peroxidase amplification step. The method measures ECP in the range of 15–1,000 pg/ml. The ECP purified from extracts of human blood eosinophils was used as standards. Before measurement, the standard and urine extracts were diluted in sample buffer (0.1% Tween 20, 0.1% CTAB, 20 mM EDTA, 0.2% human serum albumin, and 0.1% NaN_3 in phosphate-buffered saline, pH 7.4).

Ultrasound examination. Full ultrasound examination of the urinary tract was performed on the children using an Aloka (Tokyo, Japan) SSD-500 3.5 MHz convex sector scanner powered by a portable generator. A detailed report on the grading of the urinary tract pathology and its resolution and reappearance after treatment is published in a separate paper.[25] Briefly, overall pathology included any lesion of the urinary bladder, the ureters, and the kidneys. Bladder pathology was graded as follows; normal: no lesions recorded; mild: a single wall enlargement (≥5 mm) or wall irregularity; moderate: wall enlargement, wall irregularities on multiple sites, and/or 1 mass or 1 polyp; severe: multiple masses and/or polyps. No calcifications were recorded.

Treatment. Following the baseline survey, all infected pupils received a single dose of 40 mg/kg of praziquantel for treatment. The original study design to follow-up the regression and reappearance of morbidity over a 24-month period could not be maintained because 52 of the reinfected children developed severe urinary tract pathology 18 months after initial treatment. These children were re-treated immediately. The remaining children were treated at the 24-month follow-up survey. This paper presents the results for up to the 18th month following initial treatment.

Calculation of diagnostic values. The diagnostic values in relation to infection as measured by egg excretion of different levels of ECP, different grades of microhematuria, and a single egg count were calculated using data from a single urine examination. Maximal egg count data were used as reference regarding infection status. The diagnostic values in relation to bladder pathology of different ECP levels, different grades of microhematuria, single egg counts, and maximal egg counts were calculated using the results from the ultrasound examination as a reference.

Diagnostic values were calculated according to the following definitions: sensitivity = the fraction of a true positive (TP) among diseased (TP/TP + false negative (FN)); specificity = the fraction of a true negative (TN) among healthy (TN/TN + false positive (FP)); positive predictive value (PPV) = the fraction of diseased individuals among those test positive (TP/TP + FP); and negative predictive value (NPV) = the fraction of healthy individuals among those test negative (TN/TN + FN). Overall efficiency of the test was calculated as the fraction of individuals correctly classified by the test (TP + TN/TP + TN + FP + FN).

Statistical analysis. Non-parametric statistical tests were used. The Wilcoxon rank sum test and the Friedman test were used for paired observations and the Mann-Whitney rank sum test and the Kruskal-Wallis test were used for unpaired observations. The coefficient of correlation was calculated as Spearman's rho. All calculations were done using SigmaStat software (Jandell Scientific, San Rafael, CA). A P value <0.05 was considered significant.

RESULTS

Diagnostic values of urinary ECP, egg counts, and microhematuria in relation to infection at baseline. Samples were collected from 529 children at the baseline survey. The prevalence of infection was 77.9%. The median intensity among infected children was 16 eggs/10 ml of urine (range = 0–2,008 eggs/10 ml) in the single urine sample and 56 eggs/10 ml (range = 1–2,016 eggs/10 ml) when calculated from the maximal egg count obtained in 3–5 consecutive urine samples. The intensity was high, with 52% having >50 eggs/10 ml, 36% >100 eggs/10 ml, and 4.8% >500 eggs/10 ml.

The ECP level in urine from infected children (median = 151.4 ng/ml, range = 0.2–3,502.0 ng/ml, n = 412) was significantly increased compared with the level in urine from uninfected children (median = 2.4 ng/ml, range = 0.2–678.0, n = 117) ($P < 10^{-6}$). The level of urinary ECP correlated significantly with the intensity of infection (r = 0.64, n = 412; $P < 10^{-6}$).

The possible use of ECP as a marker of infection was evaluated. The ECP levels between 1 and 200 ng/ml were tested to find the optimal cut-off value for ECP as a marker of infection. The progression of diagnostic values using ECP

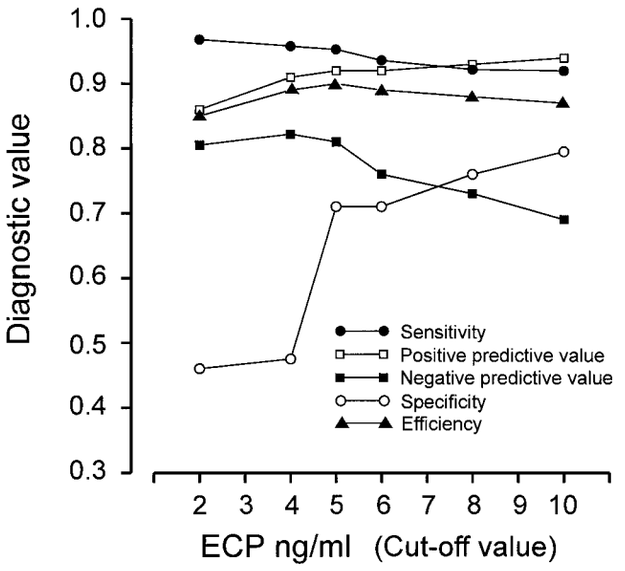


FIGURE 1. Diagnostic values of urinary eosinophil cationic protein (ECP) as a function of different ECP cut-off levels.

cut-off values between 2 and 10 ng/ml is shown in Figure 1. The optimal overall diagnostic efficiency, defined as the fraction of tested individuals correctly classified by ECP test, was reached using a cut-off value for ECP of ≥5 ng/ml. For comparison, the diagnostic values of a urinary ECP ≥5 ng/ml, different grades of microhematuria and a single egg count are shown in Table 1.

The ECP and egg count in relation to ultrasound detectable pathology at baseline. Of the 529 children present at the baseline survey, 504 had a full ultrasound examination of the urinary tract. The prevalence of overall urinary tract pathology was 67.9% (74.2% in infected children), and the prevalence of bladder pathology was 63.5% (69.2% in infected children).

The presence of ultrasound detectable pathology among infected children was highly associated with levels of urinary ECP and with the intensity of infection. In infected children with pathology in either the bladder, ureters, or kidneys (overall pathology), the median ECP level was 183.5 ng/ml (range = 0.4–3,502.0, n = 294) compared with 73.8 ng/ml (range 0.2–1,422.0, n = 102) in infected children without pathology ($P < 10^{-6}$). In children with bladder pathology alone, the median ECP level was 185.2 ng/ml (range = 0.4–3,502.0, n = 274) compared with 73.8 ng/ml (range = 0.2–1,422.0, n = 122) in infected children without bladder pathology ($P < 10^{-6}$). The same significant association was seen with the intensity of infection. Thus, the median egg count in infected children without any pathology was 5.5 eggs/10 ml (range = 0–655, n = 102) compared with 20.5 eggs/10 ml (range = 0–2,008, n = 294) in infected children with presence of overall pathology ($P < 10^{-6}$) . In infected children without bladder pathology, the median egg count was 6 eggs/10 ml (range = 0–655, n = 122) compared with 21 eggs/10 ml (range = 0–2,008, n = 274) in children with bladder pathology ($P < 10^{-6}$).

Bladder pathology was detected in 46 egg-negative children (9.1% of the study population and 42% of the egg-negative children). The median ECP level in urines from these children with ultrasound-detectable bladder pathology that could not be attributed to an active *S. haematobium* infection was 1.8 ng/ml (range = 0.2–678.0, n = 46). This figure did not differ from the ECP level in urines from non-infected children without pathology (median = 2.3 ng/ml, range = 0.2–294.4, n = 62; $P = 0.75$).

TABLE 1

Diagnostic values regarding *Schistosoma haematobium* infection of microhematuria as detected by dipstick, urinary ECP values ≥ 5 ng/ml, and egg counts ≥ 1 egg/10 ml*

	Microhematuria				ECP ≥ 5 ng/ml	Egg count ≥ 1 egg/10 ml
	Trace	+	++	+++		
Sensitivity	0.91	0.68	0.61	0.49	0.95	0.80
Specificity	0.81	0.93	0.95	0.97	0.71	1.00
PPF	0.94	0.97	0.97	0.98	0.92	1.00
NPV	0.72	0.45	0.40	0.35	0.81	0.65
Efficiency	0.88	0.74	0.68	0.60	0.90	0.88

* ECP = eosinophil cationic protein; PPV = positive predictive value; NPV = negative predictive value. Maximal egg count data from 3–5 urines collected on 3–5 consecutive days were used as the reference.

Diagnostic values regarding bladder pathology of urinary ECP and intensity of infection and hematuria at baseline. Urinary ECP values from 5 to 200 ng/ml were evaluated to find the optimal cut-off value for use of the ECP test as a marker of bladder pathology. The optimal diagnostic efficiency regarding bladder pathology was reached when using a cut-off value for the ECP of ≥25 ng/ml. This resulted in a sensitivity, specificity, PPV, NPV, and overall efficiency of 0.77, 0.5, 0.73, 0.55, and 0.67, respectively. In combination with a confirmed infection, the diagnostic performance of the same ECP cut-off value was 0.86, 0.29, 0.73, 0.48, and 0.69, respectively. The optimal diagnostic efficiency of hematuria in relation to bladder pathology was found when trace amounts, which was also the optimal cut-off level regarding infection, was used as the cut-off level. The sensitivity was high but the specificity was low. Conversely, visible hematuria had a very high specificity and PPV but a low sensitivity and overall efficiency. The diagnostic performance of microhematuria and visible hematuria, selected levels of urinary ECP (≥5, 25, and 50 ng/ml), and different levels of intensities judged from a single egg count and the maximal egg counts are shown in Table 2.

Longitudinal study. A summary of the parasitologic, pathologic, and ECP data obtained at baseline and at the post-treatment follow-up examinations conducted over a period of 18 months is shown in Table 3. A marked reduction in prevalence and intensity of infection was observed at the 2- and 4-month post-treatment surveys. The decrease in intensities was reflected in a decrease in the ECP level among infected individuals. During the same period, the prevalence of ECP levels ≥25 ng/ml and ≥5 ng/ml also decreased, reaching their minimum levels 4–6 month post-treatment. Resolution of pathology occurred more gradually, reaching a minimum level 6 months post-treatment. Thereafter, ultrasonographically detectable pathology reappeared, apparently as the children became reinfected. The parasitologic data show that egg counts due to reinfection started to increase

TABLE 2

Diagnostic performance regarding bladder pathology at the baseline examination using different cut-off values of hematuria, urinary ECP, and different cut-off values of intensities of infection judged from a single egg count examination or judged from the maximal egg count in 3–5 consecutive urine examinations*

Bladder pathology Prevalence = 63.5% n = 504		Sensitivity	Specificity	PPV	NPV	Efficiency
Microhematuria	≥ trace	0.81	0.36	0.69	0.54	0.65
	≥ +	0.65	0.64	0.76	0.51	0.64
	≥ ++	0.58	0.70	0.77	0.49	0.62
	≥ +++	0.46	0.76	0.77	0.45	0.57
Visible hematuria		0.21	0.93	0.84	0.40	0.46
Egg count (eggs/10 ml)	≥1	0.74	0.47	0.71	0.51	0.64
(single urine)	≥50	0.29	0.87	0.80	0.41	0.51
	≥200	0.07	0.97	0.82	0.37	0.40
Maximal egg count (eggs/10 ml)	≥1	0.85	0.57	0.69	0.57	0.67
(3–5 urines)	≥50	0.49	0.74	0.77	0.46	0.58
	≥200	0.15	0.93	0.81	0.39	0.44
ECP (ng/ml)	≥5 ng/ml	0.87	0.31	0.69	0.58	0.66
	≥25 ng/ml	0.77	0.50	0.73	0.55	0.67
	≥50 ng/ml	0.68	0.60	0.75	0.52	0.65

* For definitions of abbreviations, see Table 1.

4–6 months post-treatment. Significant correlations between ECP levels and egg counts were found at all examinations: r = 0.64, 0.50, 0.50, 0.53, 0.59, and 0.69 at baseline and at the 2-, 4-, 6-, 12-, and 18-month follow-up examinations, respectively ($P < 10^{-6}$).

A cohort of 214 children was present during all the examinations. The levels of ECP in samples from infected and non-infected children, prevalence of samples with the diagnostic ECP cut-off levels ≥5 ng/ml and ≥25 ng/ml, and their relationship to the post-treatment resolution/reappearance of bladder pathology are shown in Figure 2. The progression of these parameters closely paralleled the pattern seen in the cross-sectional data from the entire study population. A marked reduction in prevalence of infection was seen 2 months after treatment that slowly reached its minimum at the 4-month follow-up. High-intensity infections were almost non-existent 2 months post-treatment. The reduction in intensities was paralleled by a decrease in the levels of ECP among infected children. Treatment was also followed by a steep decrease in the prevalences of samples with ECP levels ≥5 ng/ml and ≥25 ng/ml but not to the same extent as the decreases in prevalences of infection and high-intensity infections. As with the cross sectional study, the resolution of bladder pathology occurred more slowly, reaching its minimum level 6 months post-treatment. The prevalences of infection, high-intensity infections, and samples with ECP levels ≥25 ng/ml started to increase between 4 and 6 months post-treatment, i.e., preceding reappearance of pathologic bladder lesions by at least 2 months.

Grading of bladder pathology in relation to ECP and egg counts. The relationship between severity of bladder lesions detected by ultrasonography and urinary ECP at the baseline survey and at the 12 and 18 months post-treatment follow-up surveys is shown in Figure 3. Among infected children, a clear and significant relationship between severity of ultrasound-detectable bladder pathology and urinary ECP

TABLE 3

Parasitologic, pathologic, and ECP data obtained at the baseline examination and at the 2, 4, 6, 12, and 18 months post-treatment follow-up examinations*

	Baseline	2 months	4 months	6 months	12 months	18 months
n	504	463	384	380	423	422
Prevalence of infection (3–5 urines)	78.6%	25.1%	21.9%	27.1%	53.6%	70.1%
Maximal intensity	56	3	4.5	9	15	25
(eggs/10 ml, median and range)	1–2,016	1–237	1–802	1–613	1–1,316	1–1,625
Prevalence of high intensity infections						
(≥ 50 eggs/10 ml)	41.1%	1.1%	0.8%	3.2%	13.0%	25.3%
Prevalence of infection (single urine)	66.1%	15.5%	10.9%	13.6%	36.6%	54.0%
Intensity (single urine)	16	1	0	1	4	6
(eggs/10 ml, median and range)	0–2,008	0–237	0–802	0–406	0–840	0–1,625
Prevalence of overall pathology	67.8%	33.53%	20.1%	12.9%	25.5%	35.8%
Prevalence of bladder pathology	63.5%	28.9%	15.6%	8.6%	19.6%	31.8%
Prevalence of ECP ≥ 5 ng/ml	80.9%	36.1%	31.8%	32.4%	58.9%	73.2%
Prevalence of ECP ≥ 25 ng/ml	66.9%	14.7%	13.0%	15.5%	35.2%	55.4%
ECP, ng/ml (median and range),	2.1	1.4	1.0	1.0	1.4	1.6
noninfected children	0.2–678.0	0.2–151.0	0.2–444.0	0.2–228.0	0.2–186.0	0.2–676.0
ECP, ng/ml (median and range),	149.0	22.1	19.7	15.6	38.6	105.8
infected children	0.2–3,502.0	0.2–618.0	0.2–514.	0.2–556.0	0.2–1,934.0	0.2–32,410.0

* ECP = eosinophil cationic protein.

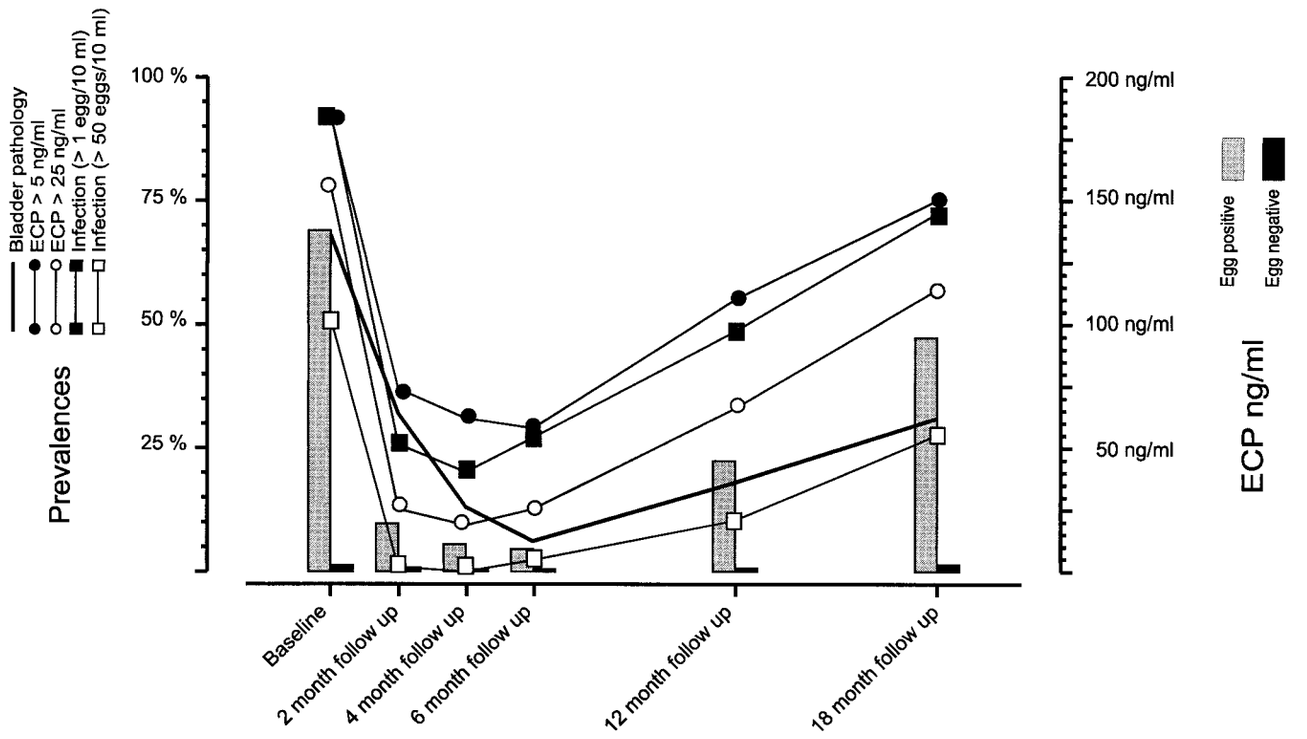


FIGURE 2. Prevalences of bladder pathology, infection, high-intensity infections, and eosinophil cationic protein (ECP) levels ≥5 and ≥25 ng/ml in a cohort of 214 children present at the baseline examinations and during all follow-up examinations. Bars indicate the median ECP levels in samples from infected and noninfected children.

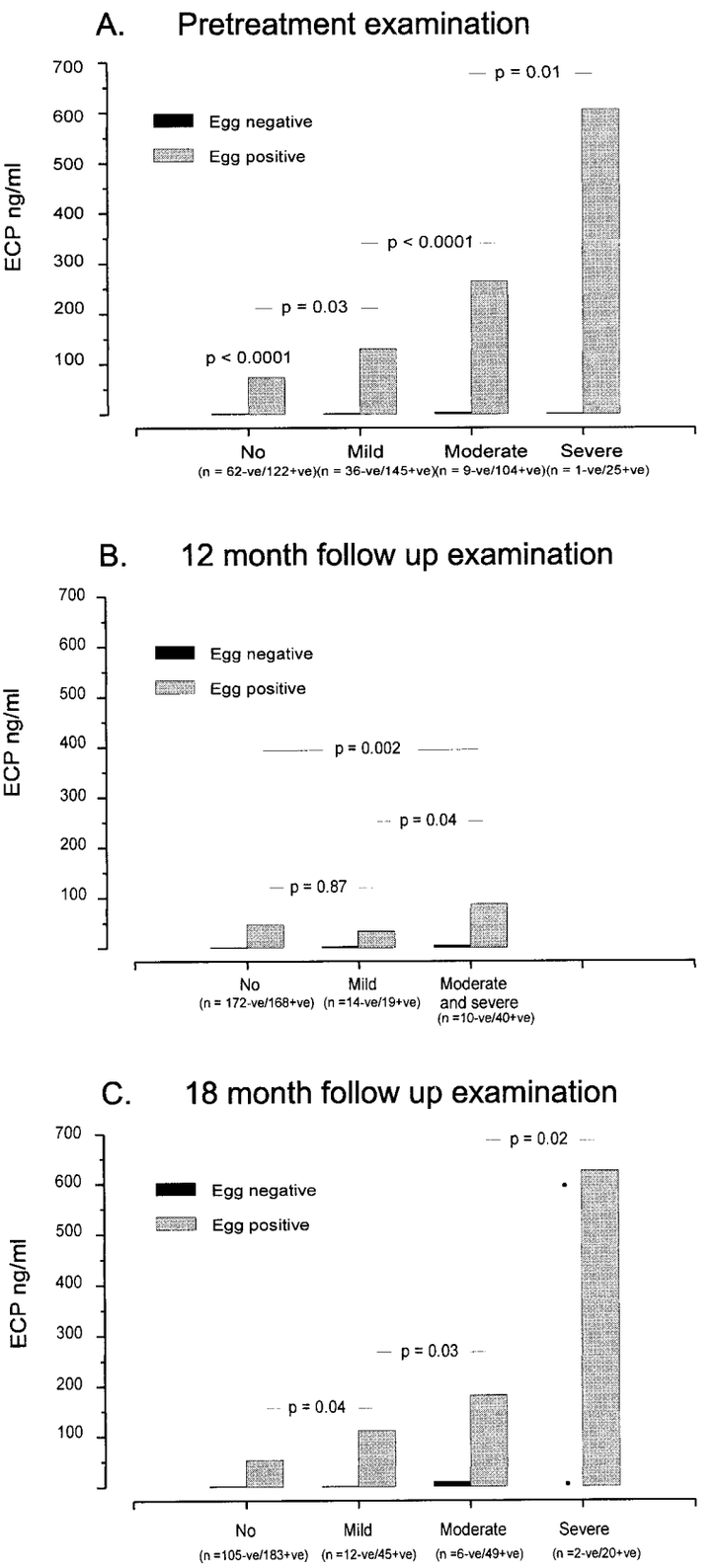
levels was seen at the baseline examination and particularly at the 18-month post-treatment. At the 12-month survey, where bladder pathology had started to reappear, a significant difference in ECP levels was found only between the group of infected children with moderate or severe pathology and the children without pathology or with only mild pathology. No difference in ECP levels was found between infected children without pathology and children with only mild pathology ($P = 0.87$). In general, the levels of ECP were significantly reduced among infected children irrespective of the presence of pathology compared with the levels in the corresponding pathology classes at the baseline and at the 18-month examination.

Egg counts were less sensitive in discriminating between the bladder pathology classes. At baseline, single and maximal egg counts could discriminate only between mild and moderate pathologies ($P = 0.013$ and 0.015) but not between no and mild bladder pathologies or between moderate and severe bladder pathologies ($0.6 > P > 0.14$). At the 12-month follow-up, single egg counts were not able to discriminate between any of the pathology classes, not even the group with no pathology compared with the groups with moderate or severe pathology ($0.87 > P > 0.27$). When the maximal egg count was used, the only significant difference between the pathology classes at the 12-month follow-up was between children with no pathology and the combined group of children with moderate or severe pathologies ($P = 0.005$). At the 18-month follow-up maximum egg counts were able to discriminate between all the pathology classes ($0.044 > P > 0.006$), whereas single egg counts discriminated only between mild and moderate bladder pathologies ($P = 0.04$).

DISCUSSION

The results from the present study provide evidence that indirect assessment of eosinophiluria using ECP is a useful indirect marker of infection and more importantly a promising direct marker of bladder morbidity, reflecting the local inflammatory host response of the bladder wall associated with urinary schistosomiasis.

Urinary ECP levels among *S. haematobium*–infected Tanzanian school children were highly and significantly increased compared with the levels of ECP in urines from uninfected children, and significant correlations between urinary ECP levels and urinary egg counts were observed at all examinations. These findings are in agreement with our previous results from a small study in Kenya[24] and with other studies describing high levels of eosinophiluria in *S. haematobium* infections.[20–23] Due to the close association between urinary ECP levels and infection status, we evaluated the diagnostic performance of the ECP test in comparison with detection of microhematuria using dipstick and a single urinary egg count determination. A urinary ECP level ≥5 ng/ml was found to be the optimal diagnostic cut-off value regarding infection. Using this cut-off value, the overall diagnostic performance of the ECP test was comparable with that of a single egg count examination or detection of trace



Grading of ultrasound detectable bladder pathology

amounts of microhematuria using the Hemastick method. Both the ECP test and the dipstick were more sensitive than a single egg count examination. The good diagnostic performance of the ECP test is consistent with the results of a study from Sudan that evaluated the use of differential urinary leukocyte counts as a diagnostic tool.[23] Using a threshold value for eosinophiluria of more than 5% of the urinary leukocytes, the sensitivity, specificity, and positive predictive values were 0.80, 0.86, and 0.82, respectively, which in their study were higher when compared with the corresponding values obtained using proteinuria, hematuria, or leukocyturia.[23] The good diagnostic performance of the Hemastick in our study supports its use as a simple and easy field applicable indirect test for infection applied in operational control programs at the community level. The ECP test could be a valuable supplement in providing information on the specific perioval eosinophilic inflammation and thus a combined marker of both infection and morbidity.

Control of schistosomiasis-associated morbidity is a major objective in schistosomiasis control programs.[4] To reach this objective, a better understanding of the pathologic process and objective and standardized measures of morbidity are necessary. Ultrasound examination of the urinary tract provides visible evidence of pathology and since its introduction in assessment of *S. haematobium*-related morbidity it has been applied both at hospitals as well as in studies at the community and school levels involving a large number of individuals.[7,12,13,16,25,28,29] These studies have shown that ultrasound-detectable pathology correlates with other measures of morbidity including urinary egg counts, hematuria, proteinuria, and leukocyturia. However, ultrasound does not detect the early inflammatory reactions or early pathologic changes of the bladder wall,[17] and despite that the intensity of infection is a major factor responsible for development of pathology, disease sequelae between individuals with the same intensities may vary.[2]

In the present study, the presence of visible pathologic changes detected by ultrasound was significantly associated with both increased levels of urinary ECP, as well as the intensity of infection. When an optimal diagnostic ECP cut-off value ≥25 ng/ml was used in relation to *S. haematobium*-related bladder pathology, the ECP test was a better diagnostic tool than a single egg count examination, and comparable with the use of egg counts as judged from the maximal egg counts in 3–5 consecutive urine examinations. In addition, the diagnostic efficiency of the ECP test appeared to be less influenced by small variations in ECP cut-off levels compared with the diagnostic efficiency of egg counts using different egg count classes (Table 2). This implies that ECP is a more stable marker of morbidity compared with egg counts. This is reasonable since the ECP test reflects the chronic inflammatory response of the host when compared with egg output, which may vary during the day.[30,31] The notion that ECP is a more accurate marker of bladder morbidity was further supported by the finding that it was better in discriminating between the pathology classes than egg counts in general and single egg counts in particular.

At baseline and at the 18-month follow-up examination, significant differences in levels of ECP were found between classes graded with no, mild, moderate, or severe bladder pathology. However, at the 12-month follow-up examination, in which pathology had started to reappear, no significant difference in ECP levels were found between infected children with no and mild pathologies. This could be due to both the low sensitivity of ultrasound as well as the high interobserver variation in detection of mild pathology (Hatz CF, unpublished data). However, whether or not pathology is detectable by ultrasound, the ECP test clearly shows that an inflammatory process involving eosinophils is occurring. The background prevalence of ultrasound-detectable bladder pathology in the study population was high. At the baseline examination, 46 of 108 uninfected children had ultrasound-detectable bladder pathology that could not be attributed to an active *S. haematobium* infection. Interestingly, the median levels of urinary ECP were not elevated in this group. At the 12-month follow-up, the levels of ECP were reduced among the infected children irrespective of the grade of pathology when compared with the levels in the corresponding pathology classes at baseline and at the 18-month post-treatment follow-up. This could indicate that the ECP levels found at baseline and at the 18-month follow-up in many of the cases reflected a kind of maximum chronic/end stage inflammatory response, but that ultrasound-detectable pathologic changes in the bladder wall begin to develop much earlier and at a lower level of inflammation. The optimal diagnostic cut-off value regarding presence of bladder pathology (≥25 ng/ml) support this notion.

Intensity of infection is the major factor responsible for development of pathology.[9–11] A close association between the severity of the egg-induced inflammatory response or number of granulomas and eosinophiluria could therefore be expected. In this study, significant correlations between ECP levels and egg counts were found at all examinations. However, the Spearman rank correlation coefficients were not very high, indicating that the intensity of infection is not the only factor influencing the inflammatory response. Other factors such as the focality of the egg deposition in the tissues, immune status, and genetic factors of the host are also probably important factors influencing the individual response to the infection and development of pathology. In the present study, ultrasound-detectable pathology was used as a reference. Thus, the ECP could never prove itself to be better than the reference. However, it appears that the ECP could be a more sensitive marker of early morbidity, irre-

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FIGURE 3. Severity of bladder pathology in relation to the median levels of urinary eosinophil cationic protein (ECP). +ve = positive. **A,** baseline examination; **B,** 12-month follow-up examination; **C,** 18-month follow-up examination. At the 12-month follow-up examination (**B**), few children had severe pathology. Data are therefore pooled with data from children with moderate bladder pathology. At the 18-month follow-up examination, 2 children with egg-negative urines showed severe bladder pathology. These children had highly different ECP levels; these data are indicated by the dots in **C.**

spective of whether pathologic lesions are detectable by ultrasound.

Increased numbers of eosinophils in the blood and tissues are seen in association with inflammatory diseases such as allergic inflammation, inflammatory lung diseases, inflammatory bowel diseases, and immune reactions against parasitic helminths.[32] Eosinophil activation in diseases associated with fibrosis and necrosis can modulate fibroblasts activity through the release of biologically active compounds that exhibit either fibrogenic or fibrolytic activities.[32–34] In addition to its granule content of cationic proteins such as ECP, major basic protein, and eosinophil peroxidase with known toxic effects on various cells and tissues including bladder epithelium,[35,36] eosinophils are now known to have the capacity to express, store, and release an array of up to 18 different cytokines, chemokines, and growth factors, including interleukin-3 (IL-3), IL-4, IL-5, IL-6, regulated upon activation normal T lymphocyte expressed and secreted (RANTES), granulocyte-macrophage–colony stimulating factor, tumor necrosis factor-α, and transforming growth factor-β.[37] Moreover, eosinophils have been shown to express major histocompatibility class II and CD4 molecules and act as antigen presenting cells *in vitro*.[38–40] These findings imply that eosinophils have both toxic and immune regulatory functions and suggest that eosinophils may play an important role in the regulation of the perioval inflammatory reactions. Thus, eosinophils could be both directly and indirectly responsible for tissue damage and development of fibrosis in *S. haematobium* infections, as well as in the wound healing and tissue repair post-treatment. The evolution/progression of pathology, ECP levels, and prevalence and intensity of infection during the longitudinal study (Table 3 and Figure 2) gives some support to this hypothesis. Thus, high-intensity infections have almost disappeared 2 months post-treatment. After an additional 2 months, this was reflected in the prevalence of urine samples with ECP levels ≥25 ng/ml reaching its minimum at the 4-month follow-up, which is followed by further resolution of bladder pathology, which reaches its lowest prevalence 2 months later at the 6-month follow-up. A steep decrease in the prevalence of infection was seen within the first 2-month post-treatment, reaching its minimum after 4 months. A concomitant but slower decrease in the prevalence of samples with ECP levels ≥5 ng/ml was also seen. Reinfection started between the 4- and 6-month follow-ups. The accompanying small increase in samples with ECP levels ≥25 ng/ml preceded development of ultrasound-detectable pathology by at least 2 months. Both parameters increased almost in parallel during the rest of the study period.

The gap between the prevalence of infection and ECP values ≥5 ng/ml increased during the first 2–4 months post-treatment. This could be explained by both an increased number of false-negative egg samples due to the low intensities or by an increased number of ECP values ≥5 ng/ml due to some continued eosinophil activity in the bladder wall during wound healing and resolution of pathology.

Eighteen-months post-treatment, the ECP levels and levels of severe pathology had nearly reached the pretreatment levels, suggesting that mass treatment should be performed on a yearly basis if development of pathology is to be prevented or kept at a low level. Alternatively, treatment every 18 months will still keep the prevalence of pathology at a lower level than the pretreatment level, but will allow more cases with severe pathology to develop.

If eosinophils are not just ''innocent bystanders'' but are actively involved and partly responsible for later development of more severe pathology, it is possible that the ECP could be used to predict the risk of later development of more severe pathology. We attempted to test this hypothesis by looking at data from children who developed pathology during a 6-month period following reinfection. Unfortunately, the study design did not allow for a detailed analysis of this question because some children appeared to become infected and developed pathology during/within the 6-month period. Interestingly, however, we traced a small group of children with egg-negative urines (either at the 6- or 12-month follow-up) who became egg positive 6 months later. Those who developed pathology during that period had a significantly increased urinary level of ECP 6 months earlier, despite the egg-negative urines. This could be explained by early, undiagnosed, low-intensity infections with eggs being lodged in the tissue and inducing a perioval inflammatory reaction. Studies with closer intervals between follow-up examinations during the period of reinfection are needed to address this question in detail.

Diagnostic values of a test result are influenced by a number of variables including the prevalence and intensity, which should be taken into consideration.[41] The prevalence of infection and morbidity in this study population was high and the diagnostic performance of the ECP test should therefore be evaluated in other endemic settings and in other age groups. Hagan[42] has pointed out some of the limitations of the methods used to generate data on prevalence and intensity of infection, which may be overcome only with improved or alternative sampling techniques. In the case of urinary ECP, it is obvious that calculation of the total amount of excreted ECP in a single spot of urine or in a 24-hr urine sample would give a more accurate estimate of the inflammatory status of the bladder wall mucosa and thus morbidity both at the community and individual level.

Monitoring of the ongoing perioval inflammatory response of the host using ECP may be a more sensitive and accurate marker of morbidity in *S. haematobium* infections than an indirect measure such as the egg output or direct assessment of gross pathologic changes using ultrasound. In conclusion, indirect assessment of eosinophiluria in *S. haematobium*-infected school children using ECP appears to be a new indirect marker of *S. haematobium* infection and a promising marker of bladder morbidity reflecting the inflammatory status of the bladder wall.

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REFERENCES

1. Warren KS, 1978. The pathology, pathobiology and pathogenesis of schistosomiasis. *Nature 273:* 609–612.
2. Smith JH, Christie JD, 1986. The pathology of *Schistosoma haematobium* infection in humans. *Hum Pathol 7:* 333–335.
3. Chen MG, Mott KE, 1989. Progress in assessment of morbidity due *to Schistosoma haematobium* infection. A review of recent literature. *Trop Dis Bull 86:* R1–R36.
4. World Health Organization, 1993. The control of schistosomiasis. Report of the WHO Expert Committee. *World Health Organ Tech Rep Ser 830.*
5. Dukes DC, Macdougall BRD, Orne-Gliemann RH, Davidson L, 1967. Urinary leucocyte excretion in African subjects: its relation to bacteriuria and the passage of bilharzial ova in urine. *Brit Med J 1:* 537–538.
6. Feldmeier H, Doehring E, Dafalla AA, 1982. Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. *Trans R Soc Trop Med Hyg 76:* 416–421.
7. Doehring E, Reider F, Schmidt-Ehry G, Ehrich JHH, 1985. Reduction of pathological findings in urine and bladder lesions in infection with *Schistosoma haematobium* after treatment with praziquantel. *J Infect Dis 152:* 807–810.
8. Doehring E, Ehrich JHH, Vester U, Feldmeier H, Poggensee U, Brodehl J, 1985. Proteinuria, haematuria, and leukocyturia in children with mixed urinary and intestinal schistosomiasis. *Kidney Int 28:* 520–525.
9. Abdel-Salam E, Ehsan A, 1978. Cytoscopic picture of *Schistosoma haematobium* in Egyptian children correlated to intensity of infection and morbidity. *Am J Trop Med Hyg 27:* 774–778.
10. Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R, Sippel JE, 1978. *Schistosoma mansoni* and *S. haematobium* infections in Egypt. III. Extrahepatic pathology. *Am J Trop Med Hyg 27:* 55–57.
11. Mott KE, Dixon H, Osei-Tutu E, England EC, 1983. Relation between intensity of *Schistosoma haematobium* infection and clinical haematuria and proteinuria. *Lancet i:* 1005–1007.
12. Doehring E, Ehrich JHH, Reider F, Dittrich M, Schmidt-Ehry G, Brodehl J, 1985. Morbidity in urinary schistosomiasis: relation between sonographical lesions and pathological urine findings. *Trop Med Parasitol 36:* 145–149.
13. Hatz C, Mayombana C, de Savigny D, Macpherson C, Koella J, Degrémont A, Tanner M, 1990. Ultrasound scanning for detecting morbidity due to *S. haematobium* and its resolution following treatment with different doses of praziquantel. *Trans R Soc Trop Med Hyg 84:* 84–88.
14. King CH, Lombardi G, Lombardi C, Greebblatt R, Hodder S, Kinyanjui H, Ouma J, Odiambo O, Bryan PJ, Muruka J, Magak P, Weinert D, Mackay W, Ransohoff D, Houser H, Koech D, Siongok TKA, Mahmoud AF, 1988. Chemotherapy-based control of schistosomiasis haematobium. I. Metrifonate versus praziquantel in control of intensity and prevalence of infection. *Am J Trop Med Hyg 39:* 295–305.
15. Hatz C, Jenkins JM, Meudt R, Abdel-Wahab MF, Tanner M, 1992. A review of the recent literature on the use of ultrasonography in schistosomiasis with special reference to its use in field studies. 1. *Schistosoma haematobium. Acta Trop 51:* 1–14.
16. Doehring-Schwerdtfeger E, Kardoff R, 1995. Ultrasonography in schistosomiasis in Africa. *Mem Inst Oswaldo Cruz 90: 2:* 141–145.
17. Burki A, Tanner M, Burnier E, Schweizer W, Meudt R, Degrémont A, 1986. Comparison of ultrasonography, intravenous pyelography and cystoscopy in detection of urinary tract lesions due to *Schistosoma haematobium. Acta Trop 43:* 139–151.
18. Moore DL, Grove DI, Warren KS, 1977. The *Schistosoma mansoni* egg granuloma: quantitation of cell populations. *J Pathol 121:* 41–50.
19. Hutchison HS, 1927. The pathology of Bilharziasis. *Am J Pathol 1:* 1–27.
20. Powell SJ, Maddison SE, Elsdon-Dew R, 1965. Urinary leucocytes in Bilharzia. *S Afr Med J 39:* 165.
21. Bhatt KM, Bhatt SM, Kanja C, Kyobe J, 1984. Urinary leukocytes in bladder schistosomiasis. *East Afr Med J 61: 6:* 449–453.
22. Eltoum IA, Ghalib HW, Suliaman S, Kordofani A, Mustafa MD, Homeida M, 1989. Significance of eosinophiluria in urinary schistosomiasis. A study using Hansel's stain and electron microscopy. *Am J Clin Pathol 92:* 329–338.
23. Eltoum IA, Suliaman SM, Ismail BM, Ismail AIA. Ali MMM, Homeida MMA, 1992. Evaluation of eosinophiluria in the diagnosis of schistosomiasis haematobium: a field-based study. *Am J Trop Med Hyg 46:* 732–736.
24. Reimert CM, Ouma JH, Mwanje MT, Magak P, Poulsen LK, Vennervald BJ, Christensen NØ, Kharazmi A, Bendtzen K, 1993. Indirect assessment of eosinophiluria in urinary schistosomiasis using eosinophil cationic protein (ECP) and eosinophil protein X (EPX). *Acta Trop 54:* 1–12.
25. Hatz CF, Vennervald BJ, Nkulila T, Vounatsou P, Kombe Y, Mayombana C, Mshinda H, Tanner M, 1998. Evolution of *Schistosoma haematobium*-related pathology over 24 month after treatment with praziquantel among school children in southeastern Tanzania. *Am J Trop Med Hyg 59:* 775–781.
26. Peters P, Warren KS, Mahmoud AAF, 1976. Rapid accurate quantification of schistosome eggs via Nucleopore filters. *J Parasitol 62:* 154–155.
27. Reimert CM, Venge P, Kharazmi A, Bendtzen K, 1991. Detection of eosinophil cationic protein (ECP) by an enzyme-linked immunosorbent assay. *J Immunol Methods 138:* 285–290.
28. Degrémont A, Burki A, Burnier E, Schweizer W, Meudt B, Tanner M, 1985. Value of ultrasonography in investigating morbidity due to *Schistosoma haematobium* infection. *Lancet 1:* 662–665.
29. Heurtier Y, Lamothe F, Develoux M, Docquier J, Mouchet F, Sellin E, Sellin B, 1986. Urinary tract lesions due to *Schistosoma haematobium* infection assessed by ultrasonography in a community based study in Niger. *Am J Trop Med Hyg 35:* 1163–1172.
30. Pugh RNH, 1979. Periodicity of output of *Schistosoma haematobium* eggs in urine. *Ann Trop Med Parasitol 73:* 89–90.
31. Doehring E, Feldmeier H, Daffalla AA, 1983. Day-to-day variation and circadian rhythm of egg excretion in urinary schistosomiasis in the Sudan. *Ann Trop Med Parasitol 77:* 587–594.
32. Wardlaw AJ, Moqbel R, Kay B, 1995. Eosinophils: biology and role in disease. *Adv Immunol 60:* 151–166.
33. Levi-Schaeffer F, Weg VB, 1997. Mast cells, eosinophils and fibrosis. *Clin Exp Allergy (suppl 1):* 64–70.
34. Kaufman LD, Gleich GJ, 1997. The expanding clinical spectrum of multisystem diseases associated with eosinophilia. *Arch Dermatol 133:* 225–227.
35. Fredens K, Dybdahl H, Dahl R, Baandrup U, 1988. Extracellular deposits of the cationic proteins ECP and EPX in tissue infiltrations of eosinophils related to tissue damage. *APMIS 96:* 711–719.
36. Kleine TJ, Gleich GJ, Lewis SA, 1998. Eosinophil major basic protein increases membrane permeability in mammalian urinary bladder epithelium. *Am J Physiol 275:* 93–103.
37. Lacy P, Moqbel R, 1997. Eokines: synthesis, storage and release from human eosinophils. *Mem Inst Oswaldo Cruz 92 (suppl 2):* 125–133.
38. Lucey DR, Dorsky DI, Nicholson-Weller A, Weller PF, 1989.

Human eosinophils express CD4 protein and bind human immunodeficiency virus 1 gp 120. *J Exp Med 169:* 327–332.
39. Lucey DR, Nicholson-Weller A, Weller PF, 1989. Mature human eosinophils have the capacity to express HLA-DR. *Proc Natl Acad Sci USA 86:* 1348–1351.
40. Weller PF, Rand TH, Barret T, Elovic A, Wong DT, Findberg RW, 1993. Accessory cell function of human eosinophils. HLA-DR dependent, MHC restricted antigen presentation and IL-1 alpha expression. *J Immunol 150:* 2554–2562.
41. Feldmeier H, Poggensee G, 1993. Diagnostic techniques in schistosomiasis control. A review. *Acta Trop 52:* 205–220.
42. Hagan P, 1996. Immunity and morbidity in infection due to *Schistosoma haematobium. Am J Trop Med Hyg 55 (suppl 5):* 116–120.