MAJOR ARTICLE

Intrathecal Immune Response Pattern for Improved Diagnosis of Central Nervous System Involvement in Trypanosomiasis

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Diagnosis of central nervous system (CNS) involvement in human African trypanosomiasis is crucial in determination of therapy. Cerebrospinal fluid (CSF) and serum immunoglobulin concentrations, blood-CSF barrier dysfunction, pattern of intrathecal immunoglobulin synthesis, trypanosome-specific antibody synthesis, and CSF lactate concentrations were analyzed in 272 patients with *Trypanosoma brucei gambiense* infection. As part of the 2- or 3-class immune response, the predominant intrathecal IgM synthesis was the most sensitive (95%) marker for inflammation of the brain. We propose to replace the World Health Organization (WHO) criteria (white blood cell count >5 cells/μL and presence of trypanosomes in CSF) with a new approach for stage determination in trypanosomiasis: CNS involvement is diagnosed only in patients with >20 cells/μL or with intrathecal IgM synthesis, independent of the presence of trypanosomes in CSF. Compared with the use of these new criteria, the WHO criteria incorrectly classified 49 of 234 patients in the meningoencephalitic stage and 7 of 38 patients in the hemolymphatic disease stage. We also show that trypanosomiasis-related immunoglobulin patterns are of value in differential diagnosis.

Infection with the protozoan parasite *Trypanosoma brucei gambiense* causes human African trypanosomiasis, or sleeping sickness. The infection is endemic to west and central sub-Saharan Africa and is transmitted through the bites of tsetse flies. After the infective bite, parasites initially proliferate in the hemolymphatic system (the first, or hemolymphatic, disease stage), but, as the disease progresses, the central nervous system (CNS) is invaded (the second, or meningoencephalitic, disease stage) [1, 2]. If the infected patient does not receive treatment, sleeping sickness is fatal.

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Differentiation between the hemolymphatic and meningoencephalitic disease stages is essential for selection of the optimal therapy (with minimal risk) for the patient. Pentamidine, a drug used for first-stage treatment, is relatively safe but is inefficient in the second stage, because it does not cross the blood-brain barrier to a sufficiently high extent. Melarsoprol, a drug commonly used for second-stage treatment, is highly toxic [3]. Thus far, stage determination is based on examination of cerebrospinal fluid (CSF) for white blood cell (WBC) count and the presence of trypanosomes [4].

For treatment decisions, the World Health Organization (WHO) recommends a WBC count cutoff of 5 cells/μL in CSF [4]. The national sleeping sickness control programs of Angola and Ivory Coast use an alternative cutoff, at the point of preliminary investigations, of 20 cells/μL [5, 6]. Concentration techniques for trypanosome detection are not widely applied and will not always reveal the presence of the parasite in the CSF.

The clinical relevance of the actual cutoff values for cell counts and the sheer presence of trypanosomes in CSF are subjects of long-lasting discussions: patients with slightly increased WBC counts (6–20 cells/μL) and patients with trypanosomes in CSF but with WBC counts <20/μL have been treated successfully with pentamidine [5, 6], the drug normally used for cases of infection without CNS involvement. The controversy around the current criteria for detection of CNS involvement in the disease has prompted studies to improve stage determination, in particular because of the higher risks associated with melarsoprol treatment.

Subacute and chronic inflammatory diseases of the CNS are accompanied by intrathecal synthesis of both immunoglobulins and specific antibodies [7]. Detection of intrathecal synthesis represents a powerful tool for detection of inflammatory processes of the brain. Assessment of a complete CNS immunoglobulin response pattern (IgG, IgM, and IgA synthesis and blood-CSF barrier function) can reveal a disease-related pattern that can contribute to differential diagnosis of neurological diseases: it can help to exclude a certain diagnosis or can point to an unexpected diagnosis [7].

To identify better parameters for stage determination and to recognize the trypanosomiasis-related immunoglobulin response pattern, for better differential diagnosis, we investigated blood-CSF barrier dysfunction, patterns of intrathecal immunoglobulin response, and trypanosome-specific antibody synthesis, in 272 patients with *T. b. gambiense* infection. We completed this basic CSF analysis by the determination of lactate in CSF, which is increased in many bacterial processes, such as bacterial meningitis or neurotuberculosis [8].

PATIENTS, MATERIALS, AND METHODS

Patients. CSF and serum samples were obtained for routine analysis, during standard procedures for diagnosis and stage determination of sleeping sickness. In total, paired CSF and serum samples of 272 patients with parasitologically confirmed (parasites in blood, lymph, or CSF) *T. b. gambiense* infection were examined before treatment. A first cohort of patients ($n = 70$) was diagnosed and was treated between 1996 and 1998 in the treatment centers of Daloa and Bouaflé (Ivory Coast). A second cohort of patients ($n = 164$) was treated between 1995 and 1999 in the treatment center of Omugo (northern Uganda). A third cohort of patients ($n = 38$) originated from Ivory Coast and was identified in 2000 during a medical survey near the town of Bonon and was treated in Daloa. The mean age of the patients was 29 years (range, 4–73 years), and the ratio of males to females was 1:1.

Stage determination. Stage determination was based on CSF WBC count and presence of trypanosomes in CSF, according to the current WHO criteria [4], with a subgroup of second-stage patients with 6–20 cells/μL [6]. Patients in the first stage had WBC counts 0–5 cells/μL and had no trypanosomes in CSF; patients in the early second stage had WBC counts 6–20 cells/μL and had no trypanosomes in CSF; and patients in the late second stage had >20 cells/μL or had trypanosomes in the CSF.

Cell counts were made with either a Fuchs-Rosenthal counting chamber (cohort 2) or a Malassez counting chamber (1-μL volume; cohorts 1 and 3). Detection of trypanosomes was performed after double centrifugation of the CSF [9].

Immunoglobulin, albumin, total protein, and lactate concentrations. Total IgG, IgA, IgM, and albumin concentrations, in CSF and serum, were determined on a nephelometer (Prospec; Dade-Behring); for IgA and IgM analysis, the particle-amplified assays NLatex-IgA (OQAI 11) and NLatex-IgM (OQAC 11) were used, with detection limits at 0.3 and 0.5 mg/L, respectively. Lactate concentrations in CSF were determined by Lactate paraaminoantipurine model (BioChemica; R. Greiner). Total protein concentrations in CSF were determined in duplicate by the bicinchoninic acid protein assay reagent, as described by the manufacturer (Pierce), by use of the microtiter plate protocol, with bovine serum albumin as standard. Because of insufficient sample volume, lactate concentrations could not be determined for 6 CSF samples and total protein concentration could not be determined for 1 CSF sample.

Trypanosome-specific IgG. Trypanosome-specific IgG in CSF and in serum was detected by a quantitative ELISA [10], with slight modification. As antigen, a mixture of variable surface glycoproteins of *T. b. gambiense* (LiTat 1.3, 1.5, and 1.6, at equal concentrations), in a total concentration of 2 μg/mL, was coated. For testing, wells were filled with serial dilutions of CSF (1:80–1:320) and of serum (1:19,200–1:76,800). Trypanosome-specific IgG antibody concentrations in CSF and in serum were interpolated from a standard curve and were expressed as arbitrary units, and the trypanosome-specific CSF/serum quotient Q_{sp}(IgG) was calculated.

Blood-CSF barrier function. Blood-CSF barrier function was assessed by the albumin quotient Q_{Alb} = CSF albumin/serum albumin. The age-related upper limit of the reference range was calculated as $Q_{Alb} = (4 + \text{age}/15) \times 10^{-3}$ [7].

Intrathecal humoral immune response. For evaluation of the intrathecal immunoglobulin response, the hyperbolic discrimination line, Q_{Lim}, between blood-derived values and intrathecal synthesis, for IgG, IgM, or IgA, was calculated [11] as $Q_{Lim} = (a/b) \times (Q_{Alb}^2 + b^2)^{1/2} - c$, with the following parameters: $a/b = 0.93$, $b^2 = 6 \times 10^{-6}$, and $c = 1.7 \times 10^{-3}$, for IgG; $a/b = 0.67$, $b^2 = 120 \times 10^{-6}$, and $c = 7.1 \times 10^{-3}$, for IgM; and $a/b = 0.77$, $b^2 = 23 \times 10^{-6}$, and $c = 3.1 \times 10^{-3}$, for IgA [11].

The extent of intrathecal synthesis in a single patient is calculated as intrathecal fraction (IF), with Q_{Lim}(Ig) for its empirical Q_{Alb} and its empirical immunoglobulin concentration

quotients (Q_{Ig} = CSF immunoglobulin/serum immunoglobulin), according to IF = $[1 - Q_{Lim}(I_g)/Q_{Ig}] \times 100$ in a percentage. An IF >0% was considered to be pathological in this case of statistical evaluation, in contrast with an IF >10% in the case of diagnosis of a single patient [7]. On the basis of the hyperbolic function Q_{Lim}, intrathecal IgG, IgA, and IgM synthesis was evaluated graphically, in Reibergrams [7], a process that is explained in the legend of figure 1.

The trypanosome-specific antibody index of IgG class, trypanosome-AI(IgG), was determined as $AI = Q_{sp}/Q_{Ig}$ when $Q_{Ig} < Q_{Lim}$ or as $AI = Q_{sp}/Q_{Lim}$ when $Q_{Ig} > Q_{Lim}$ [12]. Q_{Lim} was calculated with the parameters for *a*, *b*, and *c* that were given above. AI < 1.5 was considered normal, and AI ⩾ 1.5 was considered pathological [12].

In one example of a calculation of IF and AI [7, 13], we used the data of the patient with trypanosomiasis who is shown in figure 2: CSF/serum albumin, $Q_{Alb} = (149 \text{ mg/L})/(27.5 \text{ g/L}) = 5.42 \times 10^{-3}$; CSF/serum IgG, $Q_{IgG} = (224 \text{ mg/L})/(25.2 \text{ g/L}) = 8.89 \times 10^{-3}$; $Q_{Lim}(IgG) = (a/b) \times (Q^2_{Alb} + b^2)^{1/2} - c = 0.93 \times [(5.42 \times 10^{-3})^2 + 6 \times 10^{-6}]^{1/2} - 1.7 \times 10^{-3} = 3.83 \times 10^{-3}$; $IF_{IgG} = [1 - Q_{Lim}(IgG)/Q_{IgG}] \times 100\% = [1 - (3.83 \times 10^{-3})/(8.89 \times 10^{-3})] \times 100\% = 57\%$ (IF_{IgA} and IF_{IgM} were calculated similarly, with the appropriate parameters); $Q_{sp}(IgG) = 13.45\text{U}/78\text{U} = 17.24 \times 10^{-3}$; and $AI(IgG) = Q_{sp}(IgG)/Q_{Lim}(IgG) = (17.24 \times 10^{-3})/(3.83 \times 10^{-3}) = 4.5$.

Intrathecal inflammatory process. An inflammatory process of the brain is defined by a cellular or humoral immune response detectable in CSF [13]. Cell counts >20 cells/μL are regarded as definitely inflammatory, but cell counts of 5–20 cells/μL are not. Any intrathecal humoral immune reaction is considered an inflammatory process (IF > 0, AI ⩾ 1.5, $Q_{IgG} > Q_{Alb}$, $Q_{IgA} > Q_{IgG,}$ or $Q_{IgM} > Q_{IgA}$).

RESULTS

Cell Counts and Presence of Trypanosomes, in CSF

Thirty-eight patients (WBC counts 0–5 cells/μL and no trypanosomes in CSF) were classified as being the first stage of the disease. The early and late second-stage groups consisted of 53 and 181 patients, respectively. The late second-stage group had a median WBC count of 93 cells/μL (interquartile range [IQR], 22–266 cells/μL; maximum, 1430 cells/μL), and trypanosomes were observed in CSF from 157 patients. Among these 157 patients, 11 had WBC counts <6 cells/μL and 31 had cell counts of 6–20 cells/μL. The late second-stage patients with no detectable trypanosomes in CSF ($n = 24$) had, per definition, WBC counts >20 cells/μL (range, 22–1152 cells/μL; median, 46 cells/μL; IQR, 30–132 cells/μL).

CSF and Serum Protein Concentrations

Albumin. Albumin concentrations in serum samples of patients with trypanosomiasis (table 1) were below the reference range (35–55 g/L) of a normal European population [14]. The albumin concentrations in CSF were also low, but the CSF/serum quotients match the reference range of the European population [11].

The median values of the albumin CSF/serum quotient, Q_{Alb} (tables 1 and 2; figure 1) show that blood-CSF barrier function was predominantly normal (maximum $Q_{Alb} = 21.5 \times 10^{-3}$). Only 6%–8% (table 2) of the patients in the first and early second stages had a blood-CSF barrier dysfunction—that is, an increased Q_{Alb}. Only late second-stage patients had a remarkable frequency (43%) of blood-CSF barrier dysfunction, which is also reflected by an increased median Q_{Alb} value of 5.3×10^{-3}, compared with 3.07×10^{-3} for the first-stage group (table 1).

Total protein concentration in CSF. Compared with a European population with normal total protein concentrations in CSF of <500 mg/L [14], patients with trypanosomiasis had higher total protein concentrations in CSF, even before any involvement of the brain (table 1). This is a particular consequence of the high serum immunoglobulin concentrations of these patients. On the basis of receiver-operator characteristics analysis [15], total protein concentrations of ⩾750 mg/L in patients with sleeping sickness indicate blood-CSF barrier dysfunction, with 85.7% sensitivity and 82.4% specificity. The low albumin concentrations with the very high immunoglobulin concentrations in blood and in CSF change the relation of CSF albumin/CSF total protein (calculated from table 1), which drops below the normal values of 35%–85% observed in a European reference population [7].

Immunoglobulins. In serum samples, IgG and IgM concentrations were drastically increased: they were several-fold higher than were normal European reference values (table 1). For IgG, median values of 30 g/L were observed in the 3 groups, compared with a reference range of 8–18 g/L [14]. For IgM, medians of 9–12 g/L were observed, compared with a reference range of 0.6–2.5 g/L. Only IgA concentrations, with medians of 1.9–2.2 g/L (compared with a reference range of 0.9–4.5 g/L), were similar to European reference values.

Because of the high concentration of IgG and IgM in blood, IgG and IgM concentrations in CSF of first-stage patients were correspondingly high (table 1). There were no large differences in CSF immunoglobulin concentrations between first and early second stage, but, in late second stage, the IgM concentration in CSF was increased by 40-fold, compared with 3-fold for IgG and 4-fold for IgA. In first and early second stages, the CSF/serum concentration quotients were normal, that is, within the reference range below the upper hyperbolic discrimination line (table 2; figure 1). Some exceptions are discussed below. As is shown by the evaluation of CSF/serum quotients (table 2; figure 1), the higher immunoglobulin concentrations, in particular IgM concentrations, in the CSF of second-stage patients were due to additional intrathecal synthesis.

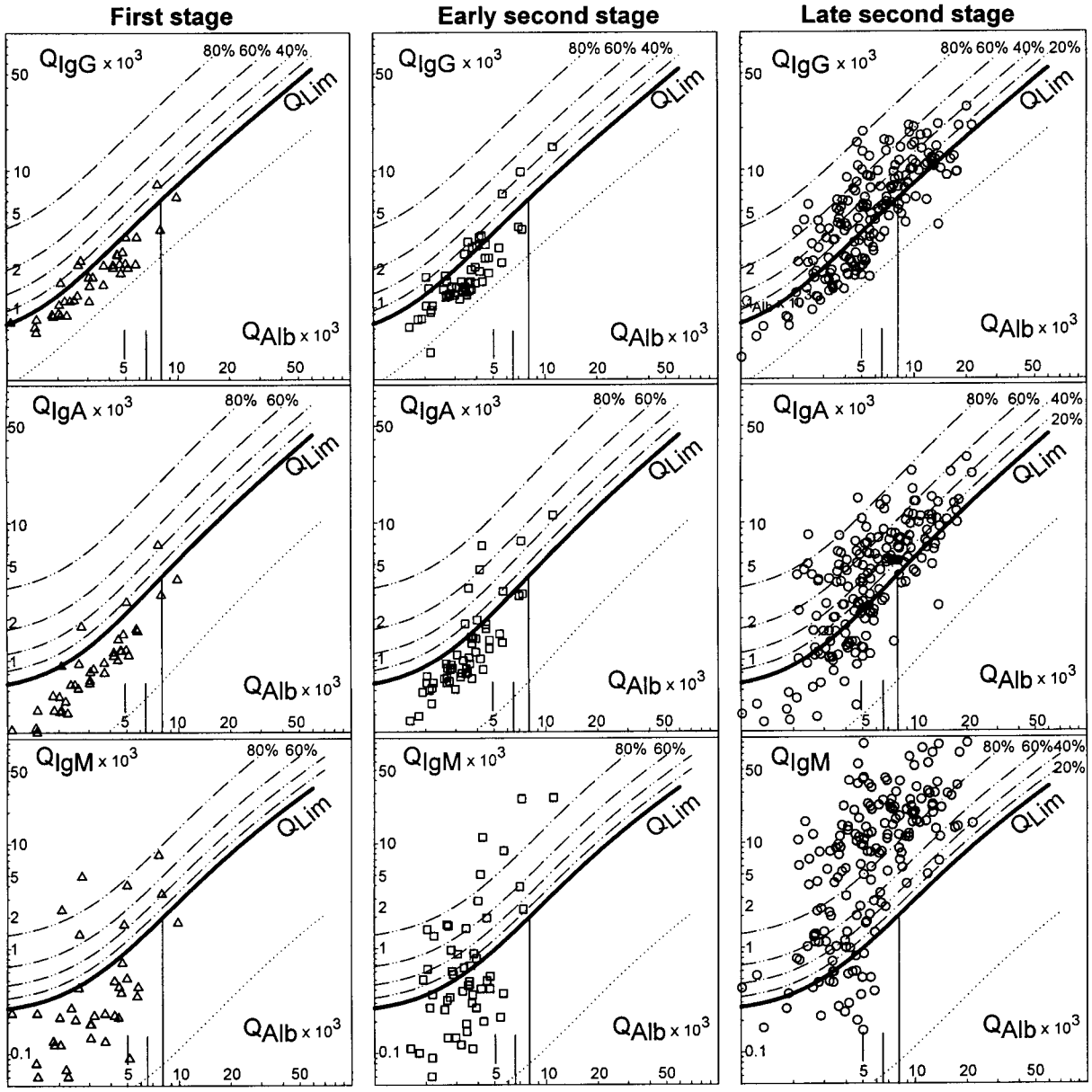

Figure 1. Quotient diagrams for IgG, IgA, and IgM (Reibergrams) [7], with data from patients infected with *Trypanosoma brucei gambiense*, in first stage *(left)*, early second stage *(middle)*, and late second stage *(right)*. Reference ranges of blood-derived IgG, IgA, and IgM fractions in CSF are between the upper discrimination line (Q_{Lim}, *bold line*) and lower discrimination line *(dotted line)*. They include 99% of a 4300-patient reference population, without intrathecal immunoglobulin synthesis [7]. Upper hyperbolic line, Q_{Lim}, represents discrimination line between brain- and blood-derived immunoglobulin fractions, in function of increasing Q_{Alb}. Values above Q_{Lim}indicate intrathecal fractions, which can be read from diagrams (*dashed lines* for 20%, 40%, 60%, 80% intrathecal synthesis). Line Q_{Lim} represents 0% synthesis. Age-dependent vertical lines indicate upper limit of reference range for age-related normal blood-CSF barrier function (in diagram, lines at $Q_{Alb} = 5$, 6.5, and 8×10^{-3} are upper limits for ages up to 15, 40, and 60 years, respectively).

Intrathecal Immunoglobulin Synthesis

The clinical relevance of the intrathecal humoral immune response of patients with trypanosomiasis is evaluated in table 2; intrathecal fractions in CSF of IF_{IgG}, IF_{IgA}, IF_{IgM}, trypanosome-AI(IgG class), and blood-CSF barrier function (Q_{Alb}) are included. The distribution patterns of the IgG, IgM, and IgA quotients, with reference to Q_{Alb}, are summarized for patients with trypanosomiasis, in quotient diagrams (figure 1). Values above the hyperbolic discrimination lines, which are between blood- and brain-derived immunoglobulin fractions [11], indicate intrathecally synthesized fractions, in percentages of total CSF concentration of the single immunoglobulin.

Of the 38 first-stage patients, 7 (18%) had intrathecal immunoglobulin synthesis: a 3-class immune reaction was ob-

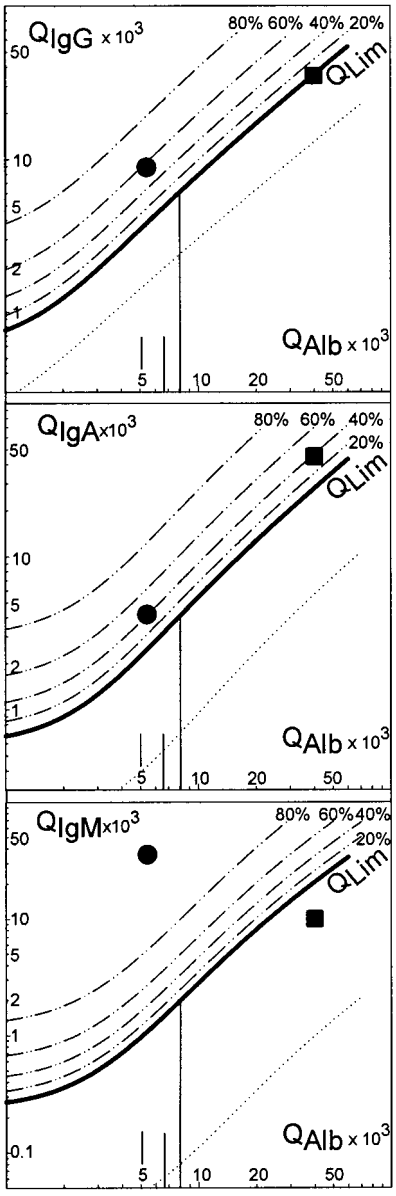

Figure 2. Comparison of typical immunoglobulin patterns and blood–cerebrospinal fluid (CSF) barrier function of a patient with trypanosomiasis due to *Trypanosoma brucei gambiense* and a patient with tuberculous meningitis (Reibergrams) [7]. For interpretation, see legend of figure 1. *Solid circle,* Patient with late second-stage trypanosomiasis (34 years old; white blood cell (WBC) count, 139 cells/μL of CSF; CSF lactate concentration of 2.9 m*M*) with 3-class immune response but age-related normal blood-CSF barrier function ($Q_{Alb} = 5.42 \times 10^{-3}$). Intrathecal IgM fraction ($IF_{IgM} = 97\%$ of total CSF IgM) is dominant, compared with intrathecal IgG fraction ($IF_{IgG} = 57\%$) or with intrathecal IgA fraction ($IF_{IgA} = 41\%$). Trypanosome-specific antibody index (AI[IgG] = 4.5) was increased. Detailed calculation of data is given as an example in Methods. *Solid square,* Patient with tuberculous meningitis (WBC count, 450 cells/μL of CSF; pathologically increased CSF lactate concentration of 6.8 m*M*; blood-CSF barrier dysfunction; $IF_{IgG} = 0\%$, $IF_{IgA} = 38\%$ [i.e., predominant IgA synthesis], and $IF_{IgM} = 0\%$ [i.e., no intrathecal IgM synthesis]).

served in 2 (5%) patients, a 2-class reaction was observed in 3 (8%) patients (2 with IgG and IgM reaction and 1 with IgA and IgM reaction), and a pure IgM-class response was observed in 2 (5%) patients. Of the 53 early second-stage patients, only 26 (49%) had an intrathecal humoral immune response: a 3-class immune reaction was observed in 7 (13%) patients, a 2-class reaction was observed in 4 (8%) patients (3 with IgG and IgM reaction and 1 with IgG and IgA reaction), a pure IgM-class response was observed in 14 (26%) patients, and a pure IgG-class response was observed in 1 (1.9%) patient. Of the 181 late second-stage patients, 158 (87%) had an intrathecal humoral immune response: a 3-class immune reaction was observed in 100 (55%) patients, a 2-class reaction was observed in 26 (14%) patients (8 with IgG and IgM reaction and 18 with IgA and IgM reaction), and an isolated IgM-class reaction was observed in 32 (18%) patients.

There were no cases in which intrathecal IgG or IgA synthesis occurred without IgM synthesis in the first stage and late second stage. There were only 2 exceptions in the early second stage, 1 case regarding IgG synthesis and 1 case regarding combined IgA and IgG synthesis.

Usually, the IgM class response was dominant ($IF_{IgM} > IF_{IgA}$ or IF_{IgG}). The intrathecal fraction of IgG was higher than the intrathecal fraction of IgM in only 3 of 272 patients, including 2 patients with intrathecal IgG synthesis ($IF_{IgG} = 2\%$ and 6%, respectively) without IgM synthesis. The intrathecal fraction of IgA was higher than the intrathecal fraction of IgM in only 3 of 272 patients, including 1 patient with intrathecal IgA synthesis ($IF_{IgA} = 55\%$) without IgM synthesis. For the patients with intrathecal synthesis, the median IF_{IgM} value was 85% (IQR, 60%–92%; maximum IF_{IgM}, 99% of total CSF IgM) versus, respectively, 31% for IgG (IQR, 15%–51%) and 44% for IF_{IgA} (IQR, 27%–61%).

Trypanosome-Specific Intrathecal Synthesis (IgG Class)

A pathological trypanosome-AI(IgG) $\geq$1.5 was detected in 46% of all the patients: 11% of the first-stage patients, 19% of the early second-stage patients, and 62% of the late second-stage patients (table 2). The median trypanosome-AI(IgG) in AI-positive patients was 3.2 (IQR, 2.1–5.1), and the maximum was 31.6. The median trypanosome-AI(IgG) in patients with a normal AI was 0.9 (IQR, 0.7–1.1).

Sensitivities for Detection of an Intrathecal Inflammatory Process

An intrathecal inflammatory process, defined as either a WBC count >20 cells/μL or any intrathecal humoral immune response, occurred in 199 patients in this study. A cellular immune response (WBC count, >20 cells/μL) was observed in 70% and a humoral immune response (pathological IF_{IgG}, IF_{IgA}, IF_{IgM}, or trypanosome-AI[IgG]) in 99.0% ($n = 197$) of these

Table 1. Stage-related IgG, IgA, IgM, and albumin concentrations, in cerebrospinal fluid (CSF) and serum samples of patients with *Trypanosoma brucei gambiense* infection, together with their total protein concentrations, white blood cell counts, and lactate concentrations, in CSF, compared with reference range of normal European population [8, 14, 16].

Parameter	First stage	Second stage		Normal European population
		Early	Late	
Serum IgG, g/L	28.7 (23.7–35.4)	29.7 (24.1–34.8)	29.6 (24.6–35.9)	8–18
Serum IgA, g/L	2.14 (1.5–3.4)	1.91 (1.44–2.46)	2.1 (1.47–2.99)	0.9–45
Serum IgM, g/L	11.3 (8.9–18.0)	9.4 (6.8–15.0)	11.7 (7.5–19.7)	0.6–2.5
Serum albumin, g/L	30.4 (23.2–33.6)	28.1 (24.3–30.7)	27.6 (24.0–31.1)	35–55
CSF IgG, mg/L	41.6 (33.9–66.0)	46.9 (32.4–67.4)	145 (75.4–272)	<40[a]
CSF IgA, mg/L	1.9 (1.2–3.4)	1.8 (1.2–3.4)	8.6 (3.5–18.5)	<6[a]
CSF IgM, mg/L	3.3 (1.6–7.5)	4.5 (2.5–11.2)	134 (17.8–262)	<1.0[a]
CSF albumin, mg/L	77.3 (65.5–114.5)	91.8 (76.4–117)	150 (103–223)	<350[a]
CSF total protein, mg/L	537 (420–652)	338 (243–483)	787 (454–1065)	<500
CSF white blood cell count, cells/μL	0 (0–3)	10 (8–14)	93 (22–266)	<5
CSF lactate, m*M*	1.6 (1.6–1.7)	1.6 (1.4–1.8)	2.2 (1.8–2.7)	<2.1
$Q_{Alb,} \times 10^3$	3.07 (2.1–4.7)	3.45 (2.7–4.2)	5.26 (3.6–8.3)	NA[b]

NOTE. Data are median (interquartile range). NA, not applicable; Q_{Alb}, albumin quotient = CSF albumin/serum albumin.
[a] Given only for orientation; reference values are defined for CSF/serum quotients.
[b] Age-related reference values [7, 13].

patients. Intrathecal IgM synthesis occurred in 95.0% (IF_{IgM} (95.0%) > IF_{IgA} (65%) > trypanosome-AI(IgG) (63%) > IF_{IgG} (62%), calculated from table 2).

Thus, in addition to the most sensitive single parameter, IF_{IgM}, other parameters contributed only 5% sensitivity. Combination of intrathecal IgM immune response with IgG and IgA, in the quotient diagrams, improved the overall sensitivity by 1%, to 96.0%. Combination of IF_{IgM} with trypanosome-AI(IgG) improved sensitivity to 98.5%, and combination of IF_{IgM} with WBC count improved sensitivity to 96.5%.

Lactate

Thirty-three percent ($n = 89$) of the patients had slightly increased lactate concentrations (2.1–3.4 m*M*) in CSF, whereas only 6% ($n = 16$) of all patients, mainly late second-stage patients, had lactate values above the 3.5 m*M* cutoff value for bacterial meningitis or neurotuberculosis (table 1) [8]. The highest lactate concentration observed in CSF was 7.7 m*M*. Increased lactate concentrations were accompanied by high WBC counts (median, 306 cells/μL) and blood-CSF barrier dysfunction (in 15 of 16 patients with increased lactate concentrations). Only 1 early second-stage patient had a lactate concentration $\geqslant$3.5 m*M*, whereas no first-stage patients had high lactate levels.

DISCUSSION

IgG and IgM concentrations, in CSF of patients with trypanosomiasis, are very high in all disease stages, because of high concentrations in blood (table 1). The calculation of the CSF/serum quotients eliminates the impact of high blood values on the CSF concentration and enables recognition of an increased CSF concentration due to an additional blood-CSF barrier dysfunction and/or intrathecal synthesis. Comparison of absolute CSF concentrations with a reference group—even an endemic control group—would lead to wrong conclusions.

The above argument is also relevant for albumin: its blood values are low, but the CSF/serum quotient of albumin matches the European reference range. Also, this is the reason why the barrier function should be interpreted on the basis of the albumin CSF/serum concentration quotient instead of on the basis of the total protein concentration in CSF [16]. The use of total protein concentration in CSF for detection of a barrier dysfunction in trypanosomiasis is biased by the immunoglobulin blood concentrations. The upper limit of the total protein reference range in CSF should therefore be higher (750 mg/L) than that used for other diseases (500 mg/L) [14]. In any case, the low cutoff values for CSF total protein concentration in trypanosomiasis that have been proposed by the WHO (370 mg/L) should be increased [4].

In patients with sleeping sickness, we found a 2–3-class immunoglobulin response (figure 1) with a predominant IgM synthesis in the CNS ($IF_{IgM} > IF_{IgA}$ or IF_{IgG}). In particular, the intrathecal synthesis of IgM is more frequent than that of other immunoglobulins (table 2). Intrathecal IgM synthesis occurred in 95% of the patients with an intrathecal inflammatory process. Combination of IF_{IgM} with trypanosome-AI(IgG) improved sensitivity for detection of an intrathecal inflammatory

Table 2. Clinical sensitivity of pathological Q_{Alb}, IF_{IgG}, IF_{IgA}, IF_{IgM}, AI(IgG), or intrathecal humoral immune response, for detection of brain involvement in trypanosomiasis due to *Trypanosoma brucei gambiense,* in first, early second, and late second disease stages.

		Second stage		
			Late	
Positive for	First stage: $n = 38$; ≤5 cells/μL in CSF; T^-	Early: $n = 53$; 6–20 cells/μL in CSF; T^-	$n = 42$; ≤20 cells/μL in CSF; T^+ in 42 patients	$n = 139$; >20 cells/μL in CSF; T^+ in 115 patients
Q_{Alb}	3 (8)	3 (6)	1 (2.4)	77 (55)
IF_{IgG}	4 (11)	12 (23)	7 (17)	101 (73)
IF_{IgA}	3 (8)	8 (15)	7 (17)	111 (80)
IF_{IgM}	7 (18)	24 (45)	22 (52)	136 (97.8)
AI_{IgG}	4 (11)	10 (19)	11 (26)	101 (73)
IF_{IgG}, IF_{IgA}, IF_{IgM}, or AI(IgG)	8 (21)	28 (53)	24 (57)	137 (98.6)

NOTE. Data are no. (%) of subjects positive for specified process. AI, antibody index; IF, intrathecal fraction; Q_{Alb}, albumin quotient = CSF albumin/serum albumin; T^-, trypanosomes absent in cerebrospinal fluid; T^+, trypanosomes present in cerebrospinal fluid.

process to 98.5%, but determination of the trypanosome-AI(IgG) is confined to specialized laboratories [10]. Detection of intrathecal IgM synthesis, combined with an inflammatory cell count >20 cells/μL, has an outstanding sensitivity (of 96.5%) for the detection of an intrathecal inflammatory process in trypanosomiasis.

Regarding specificity, the differential diagnostic relevance of the whole immunoglobulin pattern, barrier function, and lactate have to be considered. The diagnosis of trypanosomiasis would be questionable with high albumin quotients, $Q_{Alb} > 25 \times 10^{-3}$, or with high lactate concentrations. Also rare are the following: a blood-CSF barrier dysfunction without intrathecal IgM synthesis (0.7%), intrathecal IgG or IgA synthesis without intrathecal IgM synthesis (0.7% or 0.4%, respectively), and an intrathecal IgG or IgA fraction higher than the intrathecal IgM fraction ($IF_{IgG} > IF_{IgM}$ or $IF_{IgA} > IF_{IgM}$, 1.1% each).

Regarding the context of differential diagnosis, we cannot exclude the presence of other neurological infections in some of the patients we studied, especially not in patients with high lactate concentrations. Neurotuberculosis, neurosyphilis, and human immunodeficiency virus (HIV) with opportunistic infections are, in areas of endemicity, relevant to other infections of the CNS. To demonstrate the discriminative power of immunoglobulin patterns, we compared a patient with trypanosomiasis in the meningoencephalitic stage with a patient with neurotuberculosis, in figure 2. Unlike African trypanosomiasis, tuberculosis presents with severe barrier dysfunction, isolated IgA synthesis, and increased lactate concentrations (>3.5 m*M*). WBC counts are in a comparable range. An HIV encephalopathy could be easily discriminated because of its dominant but weak IgG class response and its low WBC counts, but a pattern similar to trypanosomiasis could be obtained by an opportunistic infection of the brain with a 3-class immune reaction [7]. Neurosyphilis with IgG and IgM class responses and with normal lactate concentrations in CSF could also cause a pattern similar to trypanosomiasis.

Observation of the pattern shown in figures 1 and 2 may also help to identify sleeping sickness in areas in which it is not endemic. Every year, ~20 *T. b. gambiense* infections are diagnosed outside Africa (J. Jannin, WHO, personal communication). Because of low parasite numbers, trypanosomes are not readily observed, and the infection can remain unrecognized for some time [17–20]. Again, the CNS immunoglobulin pattern is not exclusive to African trypanosomiasis. A predominant IgM response is also observed in Lyme neuroborreliosis [21] and in mumps meningoencephalitis. Isolated IgM synthesis is occasionally observed in non-Hodgkin's lymphoma involving the CNS [7].

These examples, however, indicate that the immunoglobulin pattern can guide the choice of further analyses, such as detection of specific antibody synthesis, polymerase chain reaction, and detection of the causative microorganism. It should be emphasized that the humoral immune response pattern described above is for African trypanosomiasis caused by *T. b. gambiense.* No data on the CNS humoral immune response pattern in *Trypanosoma brucei rhodesiense* sleeping sickness are available or have been published.

By the integration of the humoral immune response into the definition of CNS involvement, an essential improvement of the actual WHO criteria for stage determination is achieved. The WHO criteria are based on WBC counts and presence of trypanosomes in CSF. We propose the presence of intrathecal IgM synthesis and WBC counts >20 cells/μL, independent of the presence of trypanosomes in CSF, as a modified decision base. Moreover, the discrimination between the early and late second stages of the disease vanishes. Patients with WBC counts >20 cells/μL or with intrathecal IgM synthesis have CNS involvement. In patients with WBC counts ≤20 cells/μL, without

intrathecal IgM synthesis, trypanosomiasis is limited to the hemolymphatic system. As is shown in table 3, the WHO criteria led to 56 wrong classifications among our 272 patients.

We have described 7 first-stage patients (18%) who were already showing an intrathecal IgM response (tables 2 and 3). Pentamidine relapse rates are between 7% and 16% [3], which might be attributed to improper classification. Indeed, most pentamidine relapses are observed in the second stage [22], a finding that points to the possibility that the patients were already in that stage before treatment. Unfortunately, no data on the outcome of the patients in this study were available. The outcome of pentamidine treatment among first-stage patients with an intrathecal immune response remains to be investigated; whether they should be treated with melarsoprol or another second-stage drug also remains to be determined.

In the early second-stage group, the most striking difference, with the new criteria, is the absence of intrathecal IgM synthesis in 29 of the 53 patients. This absence might mean that 55% (29/53) of the patients in this group could have been treated with pentamidine, instead of with melarsoprol, which is recommended by WHO. Successful pentamidine treatment of patients with WBC counts 6–20 cells/μL without trypanosomes in CSF has been reported [5, 6].

The late second-stage group is a mixture of 2 different groups (table 2): a group without definite signs of neuroinflammation (WBC counts $\leq$20 cells/μL) but with presence of trypanosomes in CSF and a group with definite signs of neuroinflammation (WBC counts >20 cells/μL).

Combination of both the observation of trypanosomes in CSF and WBC counts $\leq$20 cells/μL is believed to be rare, but such a combination occurred in 15% of patients (4% with 0–5 cells/μL and 11% with 6–20 cells/μL). The utility of trypanosome detection in the CSF can be questioned, because 20 of 53 patients in this group had no definite immune response in CNS (table 3). These patients might have received an unnecessary melarsoprol treatment. Indeed, successful pentamidine treatment of patients with $\leq$20 cells/μL with trypanosomes in CSF has been reported elsewhere [6].

All patients with WBC count >20 cells/μL had a cellular immune response and thus an inflammatory brain process. The correct classification of these patients in late second stage is confirmed by the intrathecal humoral immune response. An intrathecal IgM response occurred in 97.8% of patients. The 3 IF_{IgM}-negative patients with WBC counts >20 cells/μL (24, 30, and 48 cells/μL) included 1 patient (24 cells/μL) with a pathological trypanosome-AI(IgG) of 1.6.

In conclusion, whether patients without an intrathecal inflammatory response but with trypanosomes in CSF or a mild pleocytosis (WBC count of $\leq$20 cells/μL) can be treated with pentamidine should be further investigated. Although the data on the humoral intrathecal immune response are convincing, there is not yet sufficient clinical evidence of the efficacy of the suggested alternative treatment strategy. To support the data we have presented and to change the respective WHO guidelines, confirmatory data from more settings, especially statistically relevant data on the follow-up of patients treated according to the proposed criteria, will be required. Moreover, the recent development of a semiquantitative, "low-tech" method [23] for fast analysis of intrathecally synthesized IgM fraction in CSF of patients with trypanosomiasis represents an important step toward applicability for the proposed new criteria for conditions regarding analysis in the field in Africa.

Table 3. Comparison of stage determination, according to World Health Organization (WHO) criteria, and of central nervous system (CNS) involvement, according to newly proposed criteria.

New criteria	WHO First stage (n = 38)	WHO Second stage	
		Early (n = 53)	Late (n = 181)
No CNS involvement	31	29[a]	20[a]
CNS involvement	7[a]	24	161

NOTE. Data are no. of patients.

[a] According to new criteria, these patients should receive different medical treatment.

References

1. Dumas M, Girard PL. Human African trypanosomiasis (sleeping sickness). In: Vinken PJ, Bruyn GW, eds. Handbook of clinical neurology. Vol 35. Infections of the nervous system. Part III. Amsterdam: North-Holland Publishing, **1978**:67–83.
2. Greenwood BM, Whittle HC. The pathogenesis of sleeping sickness. Trans R Soc Trop Med Hyg **1980**; 74:716–25.
3. Van Nieuwenhove S. Present strategies in the treatment of human African trypanosomiasis. In: Dumas M, Bouteille B, Buguet A, eds. Progress in human African trypanosomiasis, sleeping sickness. Paris: Springer, **1999**:253–81.
4. World Health Organization. Control and surveillance of African trypanosomiasis. WHO Tech Rep Ser **1998**; 881:1–113.
5. Pépin J, Milord F. The treatment of human African trypanosomiasis. Adv Parasitol **1994**; 33:1–47.
6. Doua F, Miezan TW, Sanon SR, Boa YF, Baltz T. The efficacy of pentamidine in the treatment of early-late stage *Trypanosoma brucei gambiense* trypanosomiasis. Am J Trop Med Hyg **1996**; 55:586–8.
7. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci **2001**; 184:101–22.
8. Wellmer A, Prange J, Gerber J, et al. D- and L-lactate in rabbit and human bacterial meningitis. Scand J Infect Dis **2001**; 33:909–13.
9. Cattand P, Miezan BT, de Raadt P. Human African trypanosomiasis: use of double centrifugation of cerebrospinal fluid to detect trypanosomes. Bull World Health Organ **1988**; 66:83–6.
10. Lejon V, Büscher P, Magnus E, Moons A, Wouters I, Van Meirvenne N. A semi-quantitative ELISA for detection of *Trypanosoma brucei gambiense* specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. Acta Trop **1998**; 69:151–64.
11. Reiber H. Flow rate of cerebrospinal fluid (CSF)—a concept common

to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci **1994**; 122:189–203.
12. Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain. Clin Chem **1991**; 37:1153–60.
13. Reiber H, Otto M, Trendelenburg C, Wormek A. Reporting cerebrospinal fluid data: knowledge base and interpretation software. Clin Chem Lab Med **2001**; 39:324–32.
14. Thomas L. Clinical laboratory diagnostics. Frankfurt: TH Books, **1998**.
15. Jacobson RH. Validation of serological assays for diagnosis of infectious diseases. Rev Sci Tech **1998**; 17:469–86.
16. Andersson M, Alvarez-Cermeno J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol Neurosurg Psychiatry **1994**; 57:897–902.
17. Kirchhoff LV. Use of a PCR assay for diagnosing African trypanosomiasis of the CNS: a case report. Cent Afr J Med **1998**; 44:134–6.
18. Damian MS, Dorndorf W, Burkardt H, Singer I, Leinweber B, Schachenmayr W. Polyneuritis und Myositis bei *Trypanosoma gambiense* Infektion. Dtsch Med Wochenschr **1994**; 119:1690–3.
19. Otte JA, Nouwen JL, Wismans PJ, Beukers R, Vroon HJ, Stuiver PC. African sleeping sickness in The Netherlands. Ned Tijdschr Geneeskd **1995**; 139:2100–4.
20. Sahlas DJ, MacLean JD, Janevski J, Detsky AS. Out of Africa. N Engl J Med **2002**; 347:749–53.
21. Tumani H, Nölker G, Reiber H. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. Neurology **1995**; 45:1663–70.
22. Jonchère H, Gomer J, Reynaud R. Traitement par les diamidines de la phase lymphatico-sanguine de la trypanosomiase humaine africaine en Afrique Occidentale Française. Bull Soc Pathol Exot Filiales **1951**; 44: 603–25.
23. Lejon V, Legros D, Richer M, et al. IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. Trop Med Int Health **2002**; 7:685–92.