

RESEARCH ARTICLE

Estimation of intrathecal IgG synthesis: simulation of the risk of underestimation

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Abstract

Objective: The low level of passively diffused IgG through the blood–brain barrier is sufficient to blur the estimation of intrathecal IgG synthesis (ITS). Therefore, this estimation requires a mathematical calculation derived from empirical laws, but the range of normal values in healthy controls is wide enough to prevent a precise calculation. This study investigated the precision of various methods of ITS estimations and their application to two clinical situations: plasma exchange and immune suppression targeting ITS. **Methods:** Based on a mathematical model of ITS, we constructed a population of healthy controls and applied a tunable ITS. **Results:** We demonstrate the following results: underestimation of ITS is common at individual level but true ITS is well fitted by cohorts; Q_{IgG} increases after plasma exchange; IgG_{Loc} calculation based on Q_{lim} falsely increases when Q_{Aib} decreases; the sample size required to demonstrate a decrease in ITS increases exponentially with larger Q_{Aib} . **Interpretation:** Studies evaluating changes in ITS level should be adjusted to Q_{Aib} . Low amounts of ITS could be largely underestimated.

Introduction

Owing to the immune privilege, B cells and plasma cells are virtually absent from the normal central nervous system (CNS). Therefore, synthesis of immunoglobulins (Igs) does not occur in the normal CNS. However, cerebrospinal fluid (CSF) contains a tiny concentration of blood-borne Igs reflecting a low-rate passive diffusion of molecules through the blood–CSF barrier (BCB) to the CSF. It has long been known that although virtually all molecules may diffuse from serum to the CSF, the permeability of the BCB positively correlates with their molecular weight.¹ For example, the ratio increases from 1:205 with albumin (65 kDa) to 1:440 with IgG (150 kDa) and 1:900 with IgM (970 kDa).² Moreover, permeability of the BCB commonly increases during CNS pathologies, leading to an increase in CSF concentrations of blood-borne proteins and Igs. As a consequence, intrathecally synthesized Igs related to CNS inflammation only increase the CSF Igs concentration, which is nonnull in the basal state. Therefore, direct assaying of Igs in the CSF is obscured by a variable concentration of blood-borne Igs,

so the exact quantification of CSF Igs synthesis requires a mathematical approach taking into account BCB permeability and blood concentrations of targeted molecules.

Quantitative results basically necessitate the subtraction of a putative basal CSF IgG (explained by normal BCB permeability) from the observed abnormal CSF IgG concentration. Since this basal CSF IgG level varies greatly in individual healthy controls, calculations are based on a cut-off situated at the upper limit of the normal group. Therefore, on an individual level, quantification of intrathecal synthesis (ITS) is intrinsically underestimated by calculation. Cohort studies may minimize this pitfall by using a cut-off based on the mean instead of the upper limit of the intrathecal concentration. However, the range of the potential underestimation of ITS has never been estimated exactly with these methods.

Patients with polyclonal Ig synthesis, which remains undetected by OCB and at too low a level to increase the IgG index, may be inappropriately classified as being devoid of ITS. This is highly problematic in patients suspected of suffering from non-MS CNS autoimmune disorders like autoimmune encephalitis since basic CSF

findings (cells, OCB, IgG index) are strong supportive clues in the early tentative diagnosis, so they may be required to undergo specific analysis. Moreover, in some cases of autoimmune encephalitis reacting against an unknown antigen, no immunoblot is available to demonstrate a putative intrathecal Ig synthesis. This lack of sensitivity of nonspecific techniques to screen ITS may lead to a greater underestimation of it than commonly thought in various CNS pathologies (i.e., stroke, Rasmussen)^{3,4} and animal models of CNS autoimmunity.^{5–7} Lastly, although ITS is mainly used nowadays as a surrogate binary clue, decreasing the ITS level may be a valuable goal so the precise monitoring of ITS may become an issue.

We present for the first time a theoretical framework demonstrating and quantifying the intrinsic underestimation of intrathecal Ig synthesis with a mathematical model. Discrepancies between calculated and exact intrathecal IgG synthesis are outlined for both single patient and cohort studies. The influences of IgG level changes on plasma and ITS are examined and the consequences for future studies targeting ITS are summarized.

Theoretical Background

The problem of passive protein transfer toward the BBB

The albumin quotient (or ratio), $Q_{Alb} = [Alb_{CSF}]/[Alb_{serum}]$, is a widely used parameter of BCB dysfunction that increases with its permeability and is influenced by age and underlying CNS pathologies. Normal maximal Q_{Alb} is calculated by the formula: $(4 + \text{age}(\text{years})/15) \times 10^{-3}$ and is usually less than $10(\times 10^{-3})$. In the basal state, which is devoid of intrathecal IgG synthesis, CSF IgG levels exclusively depend on the passive diffusion of blood IgG. Therefore, the ratio $[IgG_{CSF}]/[IgG_{serum}]$ is proportional to Q_{Alb} . Supposing a linear relation, Link et al. defined the IgG index = Q_{IgG}/Q_{Alb} , with normal values <0.7 .⁸ However, this linear cut-off did not precisely take into account either normal Q_{IgG} variance or nonlinear correlation with Q_{Alb} (ITS may be under- or overestimated depending on low or high Q_{Alb}). Moreover, since Q_{Alb} increases with age, the IgG index is thought to decrease mechanically without any change in ITS.⁹ In fact, data are best fitted by an empiric hyperbolic function (the “Reibergram”),¹⁰ whose constant parameters were later improved thanks to a large dataset of 4154 control patients (supposed to be) devoid of ITS.¹¹ Basically, the notion of hyperbolic function of quotients is based on the concept of decreased CSF flow rate, with the dual effect of a decrease in CSF volume flow and an increase in time to protein transfer, and “dysfunction of the BCB” is equivalent to a reduced CSF flow rate. Therefore, hyperbolic function is the application of Fick’s laws of diffusion applied to albumin and Igs.^{11,12}

Normal basal Q_{IgG} (Q_{IgG_basal}) is variable and cannot be exactly calculated in the event of ITS

In normal patients, all the IgG molecules in the CSF are passively diffused from blood. For a given Q_{Alb} in a population of normal patients, the distribution of Q_{IgG} follows a normal law around the mean curve: $Q_{mean} = f(Q_{Alb})$ (Fig. 1A). The variance of Q_{IgG} defined by $[(Q_{Lim} - Q_{Low})/Q_{mean}]$ is about 0.91 for each Q_{Alb} .¹¹ Individual variations in the diffusion pathway and CSF flow have been given as tentative explanations of this individual Q_{IgG} variability.¹¹

In the event of ITS, the IgG concentration in the CSF is the sum of the IgG passively diffused from blood and intrathecally synthesized IgG. The relation therefore becomes:

$$Q_{IgG} = ([IgG_{CSF_passive}] + [IgG_{CSF_Loc}])/[IgG_{serum}] \\ = Q_{IgG_basal} + Q_{IgG_Loc}; \quad (1)$$

where Q_{IgG_basal} is the putative Q_{IgG} of the same patient before the onset of ITS (Fig 1B). In clinical practice, only Q_{IgG} is directly available but not Q_{IgG_basal} or $[IgG_{CSF_Loc}]$, which may only be approximated by the choice of the discrimination curve of Reiber.

The mean Q_{IgG} among the normal population (therefore the mean Q_{IgG_basal}) is defined by the Q_{mean} curve. The upper limit of the reference range, Q_{Lim} , is usually arbitrarily fixed as $Q_{mean} + 3SD$ and involves >99% of the normal population. Using this definition, intrathecal IgG synthesis is acknowledged when $Q_{IgG} > Q_{Lim}$. Q_{Lim} is commonly fixed at $Q_{mean} + 3SD$, since in the latter case only <1% of healthy controls may display a $Q_{IgG} > Q_{Lim}$, giving a very high specificity to abnormal Q_{IgG} values. A major drawback of this reference range is a loss of sensitivity in cases displaying a low level of ITS ($Q_{IgG} > Q_{IgG_basal}$ but $Q_{IgG} \leq Q_{Lim}$). In common practice, demonstration of ITS in these cases requires a CSF-restricted OCB positivity. As expected, restriction of the reference range to $Q_{Lim} + 2SD$ instead of +3SD, although increasing the risk of false positivity (4% of normal outside this range), increases the percentage of abnormal Q_{IgG} in MS cohorts by 6–10% for IgG and up to 20% for IgM.⁹

Underestimation bias of local IgG synthesis –the “silent ITS”

The true amount of intrathecally (or locally) synthesized IgG should be calculated as:

$$IgG_{Loc} = (Q_{IgG} - Q_{IgG_basal}) \times [IgG_{serum}]. \quad (2)$$

Since Q_{IgG_basal} is an unavailable parameter, a Q_{IgG_norm} replaces it, assuming $Q_{IgG_norm} = Q_{Lim}$ or Q_{mean} . As

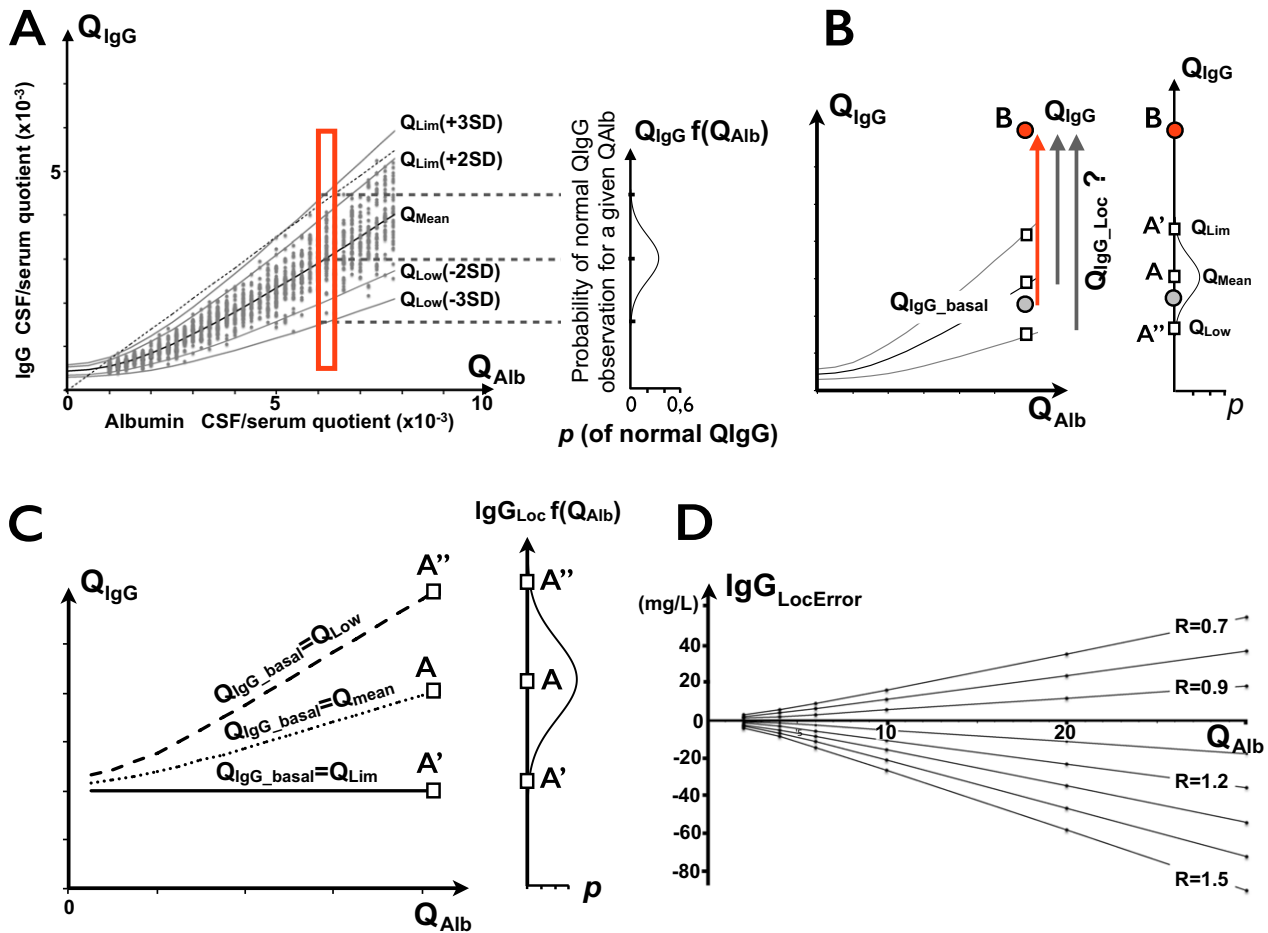


Figure 1. (A) CSF IgG passively diffused from blood to CSF in normal population. Plot of CSF/serum quotients with hyperbolic function of quotient ratios (“Reibergram”). Reference range defined by $Q_{Lim} = Q_{mean} \pm 2SD$ or $\pm 3SD$ involving 96% and 99% of normal population, respectively. Dotted line is upper normal limit (>0.7) of IgG index, intersecting Q_{Lim} curve at two points. Right insert: probability curve of basal Q_{IgG} for a given Q_{Alb} . The maximum probability is obtained for Q_{mean} . Points are obtained from a simulated healthy population. (B). Range of calculated intrathecal IgG synthesis depending on basal Q_{IgG} estimation. True IgG_{Loc} should be calculated based on normal Q_{IgG_basal} preceding disease onset (gray circle), which is unknown. In practice, Q_{IgG_Loc} , and therefore, IgG_{Loc} , strongly depend on choice of possible Q_{IgG_basal} . By raising point B as a definitely abnormal Q_{IgG} ($\gg Q_{Lim}$), the range for the ratio of intrathecally synthesized IgG depends on estimation of basal (before disease onset) Q_{IgG_basal} . In individual patients, $Q_{Lim}(+3SD)$ (point A’) is usually used as an approximation of basal Q_{IgG_basal} . However, the true Q_{IgG_basal} may be anywhere on the segment A’ – A’’, with the highest probability closest to Q_{mean} at point A. Therefore, quantitative estimation of IgG synthesis $IgG_{Loc} = (Q_{IgG} - Q_{IgG_basal}) \times [IgG_{serum}]$ is strongly influenced by arbitrary choice of Q_{IgG_basal} . (C) IgG_{Loc} depending on basal Q_{IgG} estimation. $IgG_{Loc}(Lim3SD) = 20$ mg/L, $IgG_{Loc}(mean) \approx 43$ mg/L. Therefore, the true IgG_{Loc} of $Q_{IgG}(B)$ is in the range of 20–65 mg/L. Using point B at $Q_{IgG} = Q_{Lim}(3SD) + 2$ and $[IgG_{serum}] = 10$ g/L. (D) Error range of IgG_{Loc} calculations assuming various R ratios ($R = Q_{IgG_norm}/Q_{IgG_basal}$). A ratio of 1.5 is equal to $Q_{Lim}3SD/Q_{mean}$. Calculations assume that $[IgG_{serum}] = 10$ g/L and $Q_{IgG_basal} = Q_{mean}$.

previously demonstrated, replacing Q_{IgG_basal} by Q_{Lim} confers a maximum of specificity in single patient studies, with the drawback of unavoidable underestimation of ITS. Replacing Q_{IgG_basal} by Q_{mean} is interesting since the probability of being close to the exact Q_{IgG_basal} is higher. The main drawback is the false positivity of an ITS in one half of cases (having $Q_{IgG_basal} > Q_{mean}$) and a negative result in the other half (having $Q_{IgG_basal} < Q_{mean}$), so this method of calculation is not suitable at an individual level. By contrast, this approximation is allowed and

is more precise in patient groups since differences in Q_{IgG_basal} around the Q_{mean} are compensated by the size effect of the cohort.⁹

The range of IgG_{Loc} is variable and always underestimated, depending on the Q_{IgG_basal} assumption, with the maximum of probability of IgG_{Loc} under the assumption of $Q_{IgG_basal} = Q_{mean}$. Underestimation of IgG_{Loc} is proportional to Q_{Alb} (Fig. 1B, right panel). In the case of $Q_{IgG} = Q_{Lim}(+3SD)$ ($\Delta Q_{IgG} = 0$), IgG_{Loc} , based on Q_{Lim} calculation, is estimated to be null in single individuals but

the underestimation falls within the range of 0 to 106 mg/L, in the Q_{Alb} interval 1 to 20×10^{-3} . With higher ΔQ_{IgG} , IgG_{Loc} increases, whereas the absolute value of IgG_{Loc} underestimation remains unchanged. As a consequence, this underestimated “silent” ITS can be closely approximated with a high level of probability by using Q_{mean} .

In general, the calculated amount of locally synthesized IgG may be expressed as:

$$IgG_{Loc} = (Q_{IgG_{Local}}) \times [IgG_{serum}] + IgG_{LocError}; \quad (3)$$

with $IgG_{LocError} = (Q_{IgG_{basal}} - Q_{IgG_{norm}}) \times [IgG_{serum}]$. It is therefore easy to demonstrate that the error in calculating IgG_{Loc} is proportional to $Q_{IgG_{basal}} \times (1-R)$, where R is the ratio of the $Q_{IgG_{norm}}$ and the true $Q_{IgG_{basal}}$ (i.e., $R = Q_{Lim}3SD/Q_{IgG_{basal}} \cong 1.5$). $IgG_{LocError}$ is null when $Q_{IgG_{basal}}$ is known (during simulation). On the other hand, although the range of $IgG_{LocError}$ variations remains negligible for the lowest values of R, substantial amounts are concerned for higher values of R (Fig. 1C).

The fraction of intrathecal Ig from local synthesis, IF_{IgG} (in percent), is the proportion of locally synthesized IgG among the full IgG concentration in the CSF. Theoretically, this fraction should be null only if local IgG synthesis is absent. However, based on the limitation described above and on the common assumption that $Q_{Lim} \sim Q_{IgG_{basal}}$, IF_{IgG} is calculated as:

$$IF_{IgG} = [IgG_{Loc}]/[IgG_{CSF}] = (1 - Q_{Lim}/Q_{IgG}) \times 100(\%). \quad (4)$$

Therefore, in the event of null IgG_{Loc} , although IF_{IgG} is calculated to be 0%, the maximum probability of the true IF_{IgG} in an individual patient is about 30% (0–65%) of the intrathecal fraction of IgG (Fig. 2). Interestingly, this fraction of “silent ITS” is higher for the lowest Q_{IgG} and increases along the Q_{Alb} axis. For $Q_{Alb} \geq 4$, the true IF_{IgG} is close to twice that of the calculated IF_{IgG} , and about thrice for $Q_{Alb} \geq 7$. The main consequence is that the fraction of “silent ITS” may be nonnegligible and even be major in the event of low Q_{IgG} .

Calculations are tuned by PLEX in the presence of a nonnull ITS

Healthy controls (no ITS)

PLEX lowers serum Ig levels but is not thought to modulate Ig synthesis either in the peripheral compartment or in the event of ITS. Therefore, CSF concentrations of locally synthesized Ig should not be affected. Moreover, the effect of PLEX on serum Ig levels is major but transient, depending on the synthesis rate and half-life of Ig.

Since a large fraction of CSF IgG is passively diffused from the serum, a drop in serum IgG level should decrease the CSF IgG level. On the other hand, the IgG index may mechanically increase since $[IgG_{serum}]$ is the denominator of the fraction. However, for a concentration equilibrium, $[IgG_{CSF}] = f([IgG_{serum}])$, meaning that both terms of Q_{IgG} are to be simultaneously modified. In fact, a null concentration of serum IgG should be associated with a null CSF concentration.

Diffusion from serum IgG to CSF is modeled by Fick’s law which uses a constant of diffusion thought to be a consequence of anatomical microstructures underlying the diffusion pathway and specific to each patient.¹¹ Therefore, Q_{IgG} is a constant for a given patient at a given BCB physiologic state, irrespective of $[IgG_{serum}]$. As a counterintuitive consequence, although $[IgG_{serum}]'$ decreases after PLEX, both Q_{IgG} and IgG indexes remain constant in the absence of ITS:

$$Q_{IgG_{basal}} = [IgG_{CSF_{passive}}]/[IgG_{serum}] = Q_{IgG_{basal}}' \\ = [IgG_{CSF_{passive}}]'/[IgG_{serum}]'; \quad (5)$$

so the predicted new level of CSF IgG is: $[IgG_{CSF}]' \times [IgG_{serum}]'$.

Patients with ITS

The problem gains in complexity if a substantial amount of IgG is synthesized in the CSF. Let us now suppose a constant amount of locally synthesized IgG (IgG_{Loc}). In the equilibrium state (before lowering $[IgG_{serum}]$):

$$IgG_{Loc} = (Q_{IgG} - Q_{IgG_{basal}}) \times [IgG_{serum}] = \text{constant};$$

$$Q_{IgG} = [IgG_{CSF_{passive}} + IgG_{Loc}]/[IgG_{serum}];$$

$$Q_{IgG} = Q_{IgG_{basal}} + Q_{IgG_{Loc}} \\ = [IgG_{CSF_{passive}}]/[IgG_{serum}] + [IgG_{Loc}]/[IgG_{serum}];$$

Therefore, after lowering the initial $[IgG_{serum}]$ to a lower $[IgG_{serum}]'$:

$$Q_{IgG}' = [IgG_{CSF_{passive}}]'/[IgG_{serum}]' + [IgG_{Loc}]/[IgG_{serum}]';$$

$$Q_{IgG}' = Q_{IgG_{basal}} + [IgG_{Loc}]/[IgG_{serum}]', \quad (6) \\ \text{with } Q_{IgG_{Loc}}' = [IgG_{Loc}]/[IgG_{serum}]'.$$

As a consequence, Q_{IgG}' increases proportionally to the right term of the equation 6, $Q_{IgG_{Loc}}'$. In other words, a decrease in serum IgG dramatically tunes the contrast between locally synthesized and passively diffused CSF IgG. Changes in serum albumin level, in association with

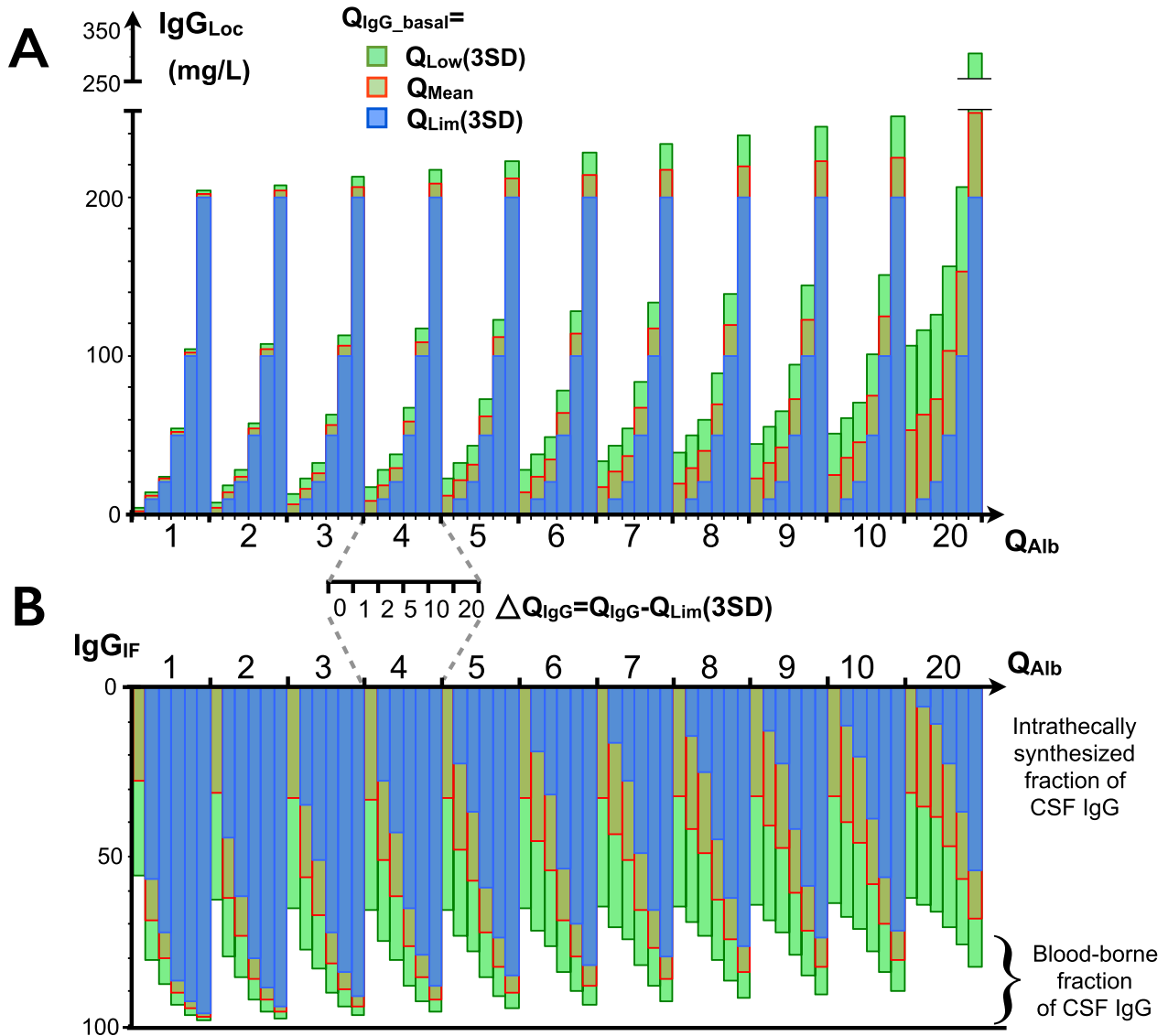


Figure 2. Influence of choice of basal IgG on estimation of intrathecal IgG synthesis. Calculations are provided for six levels of Q_{IgG} increase above Q_{Lim} (3SD) from $\Delta Q_{IgG} = 0$ to $+20$ mg/L and for each Q_{Alb} from 0 to 20×10^{-3} . Results of three formulas are displayed: formula applied to individual results: $Q_{IgG_basal} = Q_{lim}(3SD)$ (blue); formula for cohort studies, $Q_{IgG_basal} = Q_{mean}$ (red); and maximum expected value (at 99%) when $Q_{IgG_basal} = Q_{Low}$ (green). Distribution of probability of exact value is centered by red bar. (A) IgG_{Loc} . Underestimation of IgG_{Loc} is highest for the lowest increase of Q_{IgG} (0 to +2) above Q_{Lim} , and increases with higher Q_{Alb} . (B) Intrathecal fraction of IgG synthesis. The most probable intrathecal synthesized fraction of CSF IgG is far higher than estimated, especially for low ΔQ_{IgG} and high Q_{Alb} . For example, with $Q_{Alb} = 5$ and $\Delta Q_{IgG} = 1$, $I_{IgG}\%$ is probably about twice that of the expected value (47% vs 22%).

PLEX, poor physiological condition or chronic infection do not modify Q_{Alb} .^{12,13}

Closing the BCB increases Q_{IgG} although true ITS remains unchanged

The amount of passively diffused IgG from blood to CSF depends on BCB permeability, so restricting the latter mechanically decreases both Q_{Alb} and Q_{IgG} . The

corresponding point on the Reibergram shifts left of the normal curve range. In the event of significant ITS, a level of complexity is added by the dual origin of Q_{IgG} (Eq. 1), where only Q_{IgG_basal} decreases whereas Q_{IgG_Loc} remains constant. This change tunes the contrast of ITS among the IgG CSF concentrations and may shift apparently normal Q_{IgG} values outside the normal range (Fig. 3).

The IgG index always increases in response to closure of the BCB. For example, in response to a decrease

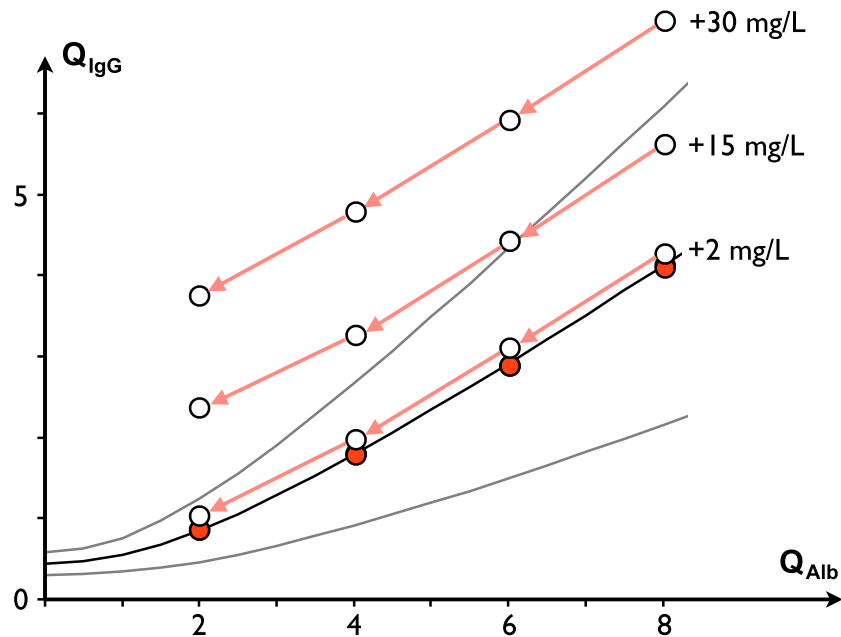


Figure 3. Evolution of Q_{IgG} with decreasing Q_{Alb} and at constant ITS. Q_{IgG_basal} (red circles) is arbitrarily fixed as equal to Q_{mean} and three examples of constant ITS levels are depicted (2, 15, and 30 mg/L). Q_{IgG} decreases as Q_{Alb} decreases. Since the variance of normal Q_{IgG} is skewed for lower Q_{Alb} , the Q_{IgG} values associated with an ITS may progressively reach an abnormal range as long as Q_{Alb} decreases. IgG index increases as Q_{Alb} decreases.

from $Q_{Alb}(\times 10^{-3}) = 8$ to 2, the IgG index increases from 0.89 to 1.92 at ITS = 30 mg/L. Therefore, an apparently low or normal IgG index may become abnormal without any increase in the former ITS in response to the normalization of the BCB (i.e., recovery of CSF flow rate).

Moreover, calculation of IgG_{Loc} , which is based on $Q_{Lim}(3SD)$ for single patient calculation, may incorrectly confirm this apparent onset of ITS, whereas calculation based on Q_{mean} is accurate.

Material and Methods

Construction of a mathematical model of intrathecal IgG synthesis

As demonstrated above, ITS is underestimated both in qualitative and quantitative terms. Although it is possible to assess it approximately, the true ITS of patients cannot be measured or calculated unless Q_{IgG_basal} is known. Therefore, using a simple mathematical model based on the abovementioned assumptions, we simulated a population of healthy controls and obtained a model of a normal Reibergram (Fig. 1). We then used this simulated healthy population to construct a cohort with a constant (tunable) ITS (C-ITS cohort) (Fig. 4).

Statistics

Random values were obtained with StatPlus (AnalystSoft Inc., v5) and used to construct the dataset. All data were processed on Excel (Microsoft Corp., v14). The chi-squared test was used to compare qualitative variables. The t-test for paired samples was used for quantitative variables since Q_{IgG} and IgG_{Loc} follow a normal rule. P value for statistical significance was set at 0.05. Calculations of sample size and statistical calculations were made with JMP (SAS Institute Inc., v8.0.2). The receiver operating characteristic (ROC) curve estimation and area under curve (AUC) (95% confidence interval, CI) were calculated with XLSTAT (Addinsoft, v19.7).

Results

Effects of variable levels of intrathecal IgG synthesis on estimated parameters of synthesis

Using the C-ITS simulated population, ROC curve estimations were obtained for various levels of ITS at different Q_{Alb} (Fig. 5A). AUC increased along with ITS but was inversely proportional to Q_{Alb} . Low amounts of ITS were poorly discriminated at high Q_{Alb} . At single patient level,

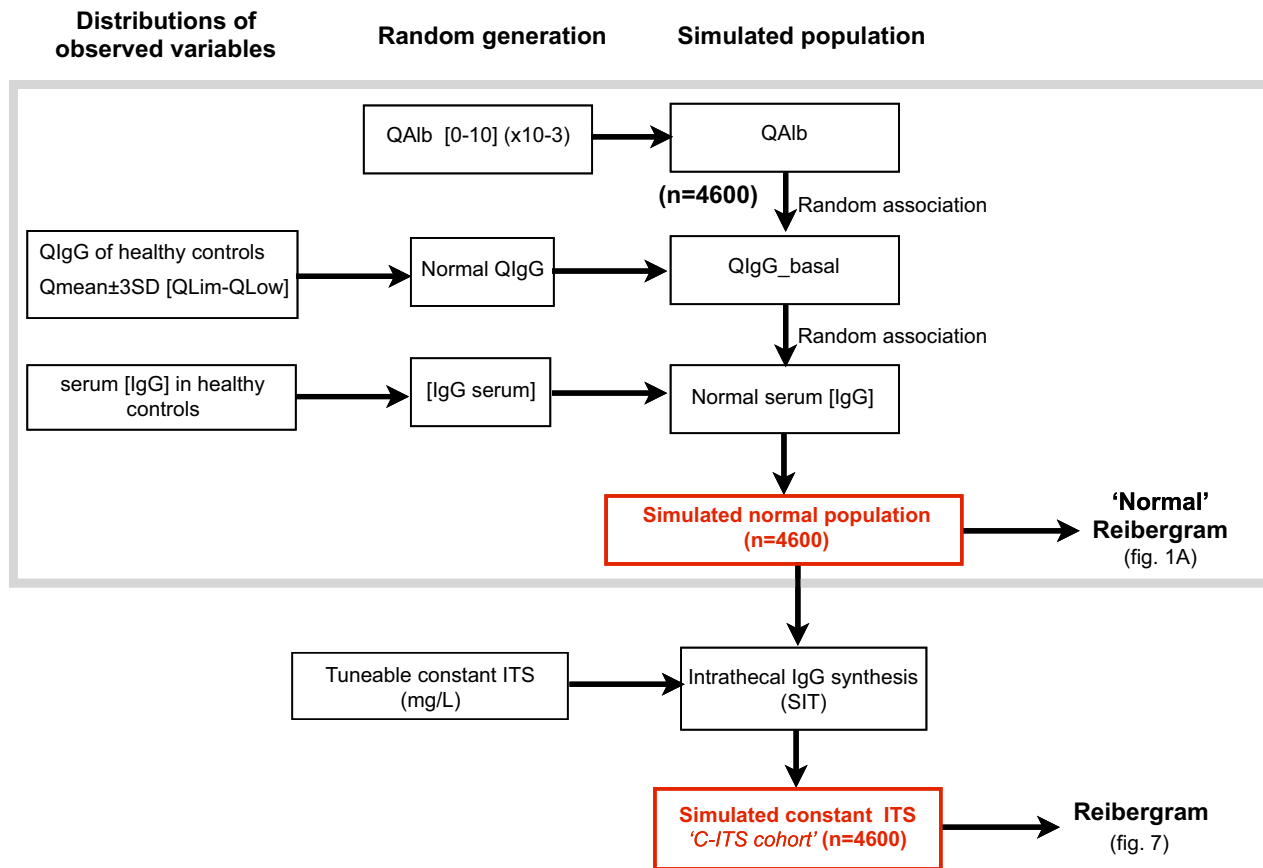


Figure 4. Construction flowchart of mathematical model simulating IgG concentration in populations of healthy controls and patients with constant intrathecal synthesis (C-ITS cohort).

estimation of true IgG_{Loc} was poor and $IgG_{Loc}(Lim)$ was highly unreliable (Fig. 5B). We compared true IgG_{Loc} values with the results obtained at a cohort level using the various possible calculations (not shown). A correct approximation of the true IgG_{Loc} was systematically obtained for calculations based on Q_{Mean} , even with IgG_{Loc} levels lower than 1 mg/L. On the other hand, estimations of IgG_{Loc} based on Q_{Lim} at 2SD or 3SD gave arbitrary results. Moreover, the precision of the results based on Q_{Mean} was independent from Q_{Alb} , whereas estimation based on Q_{Lim} was strongly biased (results not shown). However, calculation of $IgG_{Loc}(mean)$ based on the simulation of small cohorts of patients demonstrated that the speed of convergence of IgG_{Loc} was independent from the true ITS and inversely proportional to Q_{Alb} (Fig. 5C).

The proportion of abnormal Q_{IgG} strongly depended on the ITS level and the Q_{Alb} (Fig. 6). For a null ITS (normal state), about half of the Q_{IgG} were situated above the Q_{Mean} and almost none were higher than Q_{Lim} . However, at ITS levels as low as +1–5 mg/L, the distribution of Q_{IgG} was strongly biased above Q_{Mean} with the lowest Q_{Alb} . For example, for an ITS as low as +5 mg/L, 95% of

the Q_{IgG} were higher than Q_{Mean} , whereas only 13% were higher than $Q_{Lim} + 3SD$ (with $Q_{Alb} = 4 \times 10^{-3}$). As a consequence, the population bias $Q_{IgG} > Q_{Mean}$ is characteristic of a low level of ITS.

Simulation of CSF parameters after plasma exchange (PLEX)

Interestingly, lowering the IgG level tunes the IgG index and Q_{IgG} with an unexpected sensitivity. An increase in IgG index is even predicted for extremely low levels of ITS (1 mg/L) when $[IgG_{serum}]$ is decreased by more than 90%. For an ITS value of 10 mg/L (Q_{IgG} remains under Q_{Lim}), a minimal decrease in $[IgG_{serum}]$ of 20% is sufficient to turn the IgG index and Q_{IgG} into abnormal values. Results obtained for common ITS values and PLEX outcome are listed in Table 1. Note that when $[IgG_{serum}]$ was lowered even more, the fraction of IgG_{CSF} in CSF became substantial even at very low ITS rates (i.e., up to 30% of IgG_{CSF} is of local origin for ITS = 1 mg/L and PLEX rate = 90%). Interestingly, after a PLEX procedure depleting $[IgG_{serum}]$ by 90%, $[IgG_{CSF}]$ became almost a pure product of locally synthesized IgG.

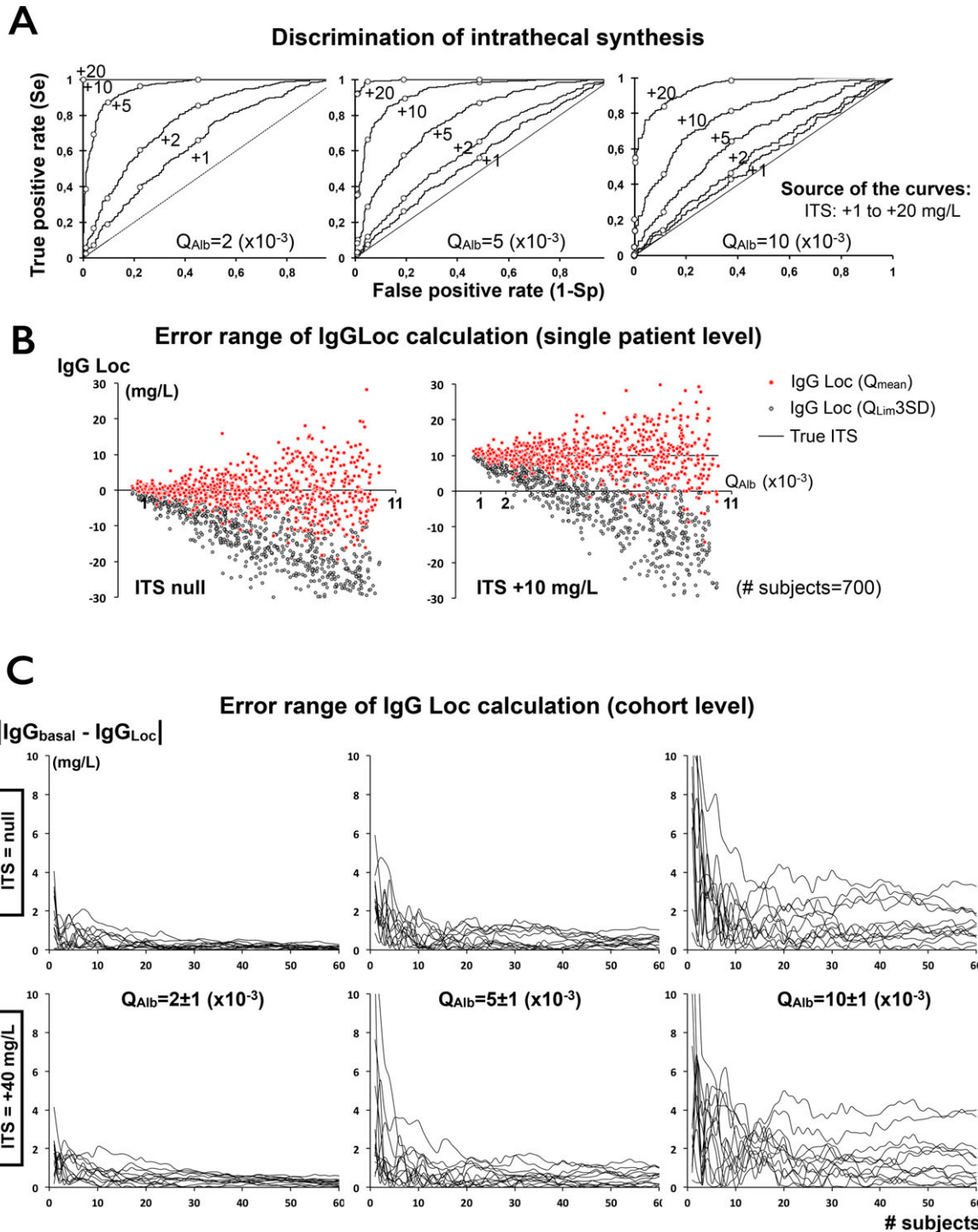


Figure 5. Estimation of intrathecal synthesis. (A) ROC curve for various amounts of ITS and different Q_{Alb} indicating sensitivity (Se) and specificity to discriminate ITS from normal Q_{IG} . Points from left to right give Se and Sp of IgG index, $Q_{Lim}(3SD)$, $Q_{Lim}(2SD)$, $Q_{Lim}(1SD)$, and Q_{mean} . All AUC except one (ITS +1 mg/L at Q_{Alb} 10) are significantly different from 0.5. Low amounts of ITS are poorly discriminated by Reibergram except at very low values of Q_{Alb} . For example, AUC of ITS +5 mg/L decreases from 0.954 (0.941–0.966) at $Q_{Alb}=2$ to 0.667 (0.624–0.710) at $Q_{Alb}=10$. (B) At single patient level, calculation of IgG_{Loc} remains unreliable, especially based on Q_{Lim} . (C) Convergence of the error range (in absolute value) of IgG_{Loc} calculation at a cohort level. Convergence toward the exact ITS value is independent from amount of ITS, but strongly depends from Q_{Alb} . Fewer than 10 patients are required to compensate extreme outliers. Formula is: $|\text{mean}(IgG_{Loc_true}) - \text{mean}(IgG_{Loc_calculated})|$. For each condition (Q_{Alb} ; ITS), up to 60 subjects are included in 12 independent simulated assays.

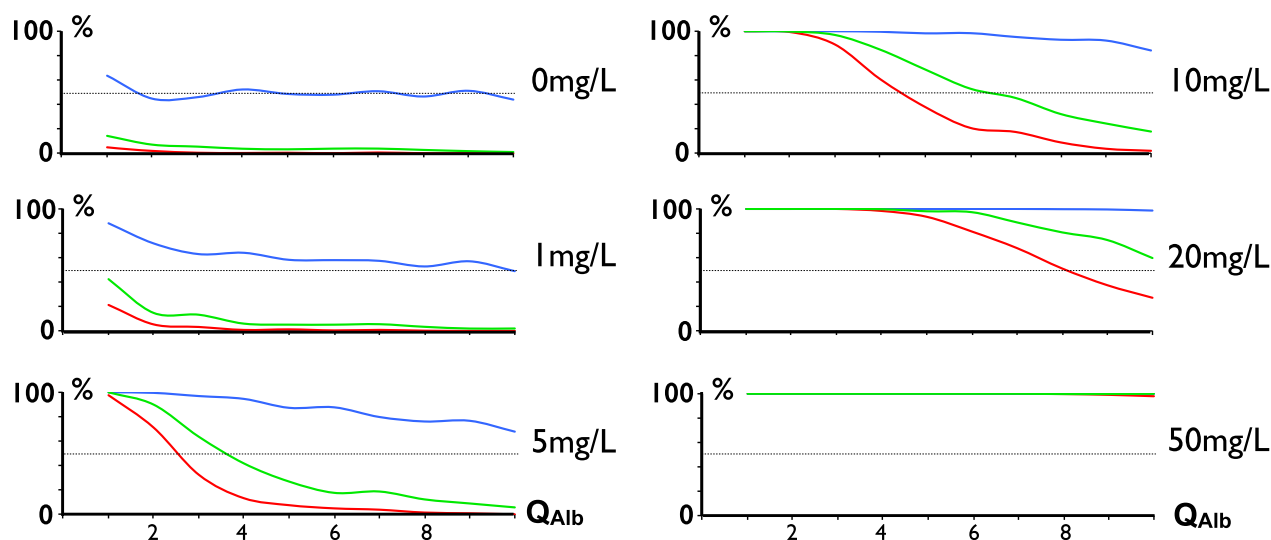


Figure 6. Proportion of Q_{IgG} (from C-ITS cohort) above curves: Q_{mean} (blue), Q_{Lim2SD} (green), and Q_{Lim3SD} (red) for various levels of ITS. At a cohort level, a shift of Q_{IgG} above the mean is more sensitive of an abnormal low-grade ITS than suggested by the proportion of patients with $Q_{IgG} > Q_{Lim3SD}$. As an extreme example, with an ITS of 5 mg/L for $Q_{Alb} = 5(\times 10^{-3})$, almost all patients are $Q_{IgG} > Q_{mean}$, whereas a few are $Q_{IgG} > Q_{Lim3SD}$.

Simulations of PLEX in the C-ITS cohort are depicted in Figure 7. PLEX increased Q_{IgG} as well as the dispersion of the values. In both examples, the ratio of the IgG index before and after PLEX increased 1.8- and 2.7-fold, respectively, after a procedure decreasing $[IgG_{serum}]$ by 80%. Note that the precision of the IgG_{Loc} estimation remained unchanged after PLEX.

Consequences for sample size determination in future studies aiming to quantify ITS variations

We provide an estimation of the minimal sample size required to demonstrate a decrease in ITS (Table 2). In the C-ITS cohort, the standard deviation of $IgG_{Loc}(mean)$ was stable whatever the fixed variations of the ITS level, but it increased collinearly with Q_{Alb} levels (1.7–8.7 in the Q_{Alb} interval $2-10 \times 10^{-3}$). Therefore, the sample size depends on the distribution of Q_{Alb} in the tested population. Sample size estimations are provided for a range of ITS variations depending on Q_{Alb} . The demonstration of very low synthesis rates remains challenging unless large cohorts can be assembled.

Discussion

A comprehensive theoretical analysis of the diffusion law from blood to CSF was proposed by Reiber et al.^{10,11} who provided an elegant mathematical formulation now widely used to quantify absolute and proportional ITS, a

criterion used as supportive evidence to diagnose autoimmune and infectious disorders. Unfortunately, this method fails to demonstrate Igs synthesis in up to 21–39% of MS cases⁹ and in up to 90% of paraneoplastic disorders.¹⁴ In the latter, a minor ITS is regularly demonstrated by techniques such as oligoclonal bands (OCB) measured by isoelectric focusing and immunoblotting against various antigens. Unfortunately, these extremely sensitive techniques provide only qualitative information so there is still a “gray zone” where ITS is qualitatively supported by OCB but remains lower than the quantitative cut-off.

Intrathecal IgG synthesis is often expressed in a binary manner where CSF is considered to be positive if either the IgG index or CSF-specific oligoclonal bands are observed. Since OCB sensitivity is higher than the two quantitative criteria (IgG index or Q_{IgG}), thorough examination of these criteria for establishing the ITS level is largely neglected. Since the pioneering work of Reiber et al., it is now known that the relation of Q_{IgG} to Q_{Alb} is a nonlinear curve.^{12,15} While methods for quantifying ITS (IgG_{Loc}) have been largely debated and several formulas have been proposed,^{16,17} the formula derived from the Reibergram remains the best approach although it provides only an approximation.¹² However, as we demonstrate above, whatever the choice of Q_{Lim} cut-off, results concerning “silent ITS” remain mostly unchanged: that is ROC curves calculated (not shown) with the procedure described by Auer et al.¹⁷ are very close to those obtained with Reiber’s formula.

Table 1. Main CSF parameters after lowering serum IgG level by plasma exchange (PLEX).

[IgG _{serum}]	True ITS (IgG _{Loc}) (mg/L)	[IgG _{CSF}] (mg/L)	IgG index	Q _{IgG} ($\times 10^{-3}$)	IgG _{Loc} (Lim) (mg/L)	True IF _{IgG} (%)	Estimated IF _{IgG} (%)
Before PLEX	0	23	0.5	2.3	0	0	0
	1	24	0.5	2.4	0	4.2	0
	5	28	0.6	2.8	0	17.9	0
	25	48	1.0	4.8	13.2	52.1	27.5
	50	73	1.5	7.3	38.2	68.5	52.4
-20%	0	18.4	0.5	2.3	0	0	0
	1	19.4	0.5	2.4	0	5.2	0
	5	23.4	0.6	2.9	0	21.4	0
	25	43.4	1.1	5.4	15.5	57.6	35.9
	50	68.4	1.7	8.6	40.5	73.1	59.3
-50%	0	11.5	0.5	2.3	0	0	0
	1	12.5	0.5	2.5	0	8.0	0
	5	16.5	0.7	3.3	0	30.3	0
	25	36.5	1.5	7.3	19.1	68.5	52.4
	50	61.5	2.5	12.3	44.1	81.3	71.7
-90%	0	2.3	0.5	2.3	0	0	0
	1	3.3	0.7	3.3	0	30.3	0
	5	7.3	1.5	7.3	3.8	68.5	52.4
	25	27.3	5.5	27.3	23.8	91.6	87.3
	50	52.3	10.5	52.3	48.8	95.6	93.3

Simulation with various levels of true ITS (from null to 50 mg/L). $Q_{IgG_basal} = Q_{mean}$ is used for the sake of clarity. Starting parameters: $Q_{Alb} = 5 \times 10^{-3}$; $Q_{IgG_basal} = 2.3 \times 10^{-3}$; $Q_{Lim}(3SD) = 3.47 \times 10^{-3}$; $[IgG_{serum}] = 10$ g/L. Results initially abnormal or becoming abnormal after PLEX are in bold. In the event of null ITS, IgG index remains normal throughout PLEX procedures, whereas if ITS is raised to 5 mg/L (see Fig. 7), IgG index becomes abnormal when basal $[IgG_{serum}]$ is reduced by 50%. Estimated IF_{IgG} increases from 0% to 52% for a 90% decrease in basal $[IgG_{serum}]$.

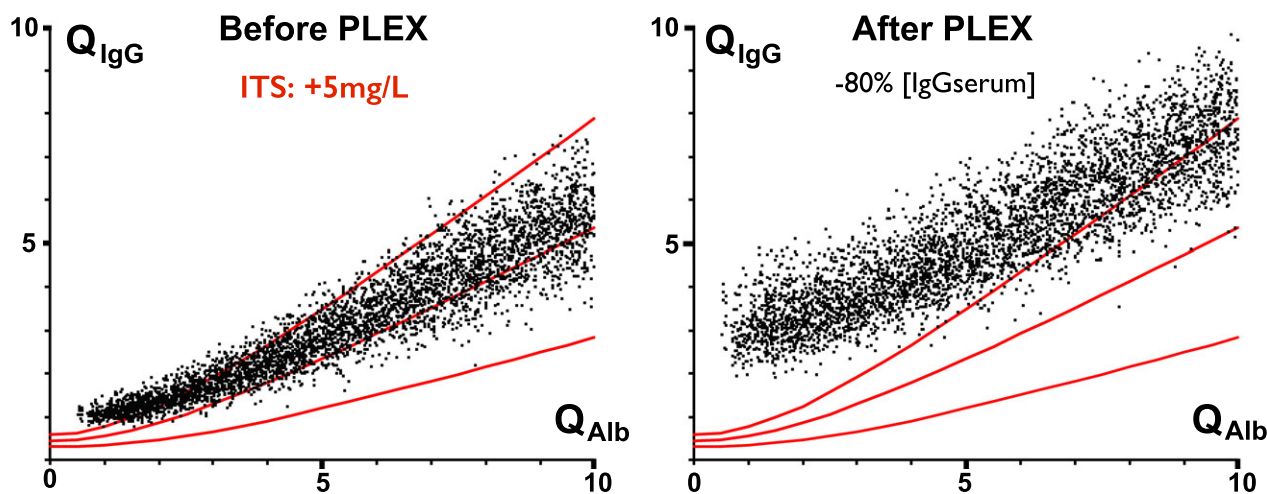


Figure 7. PLEX effect on Q_{IgG} . (Left) C-ITS population (+5 mg/L). Q_{IgG} with low Q_{Alb} are slightly increased to abnormal range. (Right) Q_{IgG} are increased and in abnormal range. Mean IgG index is increased 1.8-fold (up to $\times 3.6$).

“Silent ITS” is a common feature in MS, since up to half of the patients demonstrate Q_{IgG} below the Q_{Lim} . Therefore, follow-up of ITS in these patients could be a challenge. We demonstrate that, at an individual level, approximation of the range of ITS is limited to a statistical approach. We

show that when this formula is applied to a single patient (using Q_{Lim} as an approximation of the basal state), ITS is constantly and substantially underestimated, especially at the lowest levels of ITS. Moreover, when a single patient undergoes serial CSF analysis, successive approximations of

Table 2. Sample size estimation according to ITS changes and Q_{Alb} strata.

	$Q_{\text{Alb}} (\times 10^{-3})$			
	2	4	6	10
IgG _{Loc} SD ¹ (mg/L)	1.7	3.5	4.9	8.7
Estimated sample size for ITS variation of:				
1 mg/L	93	387	756	2379
5 mg/L	7	18	33	98
10 mg/L	4	7	10	26
IgG index (SD)				
Basal	0.42 (0.08)	0.45 (0.08)	0.48 (0.08)	0.52 (0.08)
+ITS 10 mg/L	0.94 (0.16)	0.69 (0.1)	0.65 (0.08)	0.62 (0.08)
Estimated sample size:	6	7	10	23

Sample size is estimated to detect a difference with a power of 80% at $P = 0.05$.

¹SD of IgG_{Loc} are similar whatever the level of basal ITS.

ITS may give the misleading impression of changes in ITS. For example, if Q_{Alb} is decreased by a treatment “closing” the BCB, the apparent ITS may increase although the true ITS remains unchanged. In a given patient undergoing serial CSF examinations, the apparent change in or onset of ITS should be carefully interpreted if Q_{Alb} decreases. In this case, the calculation of IgG_{Loc} should not be based on Q_{Lim} but rather on Q_{mean} , which minimizes the risk of error.

Q_{Alb} in MS patients is sometimes slightly higher than expected with age, but evolution of Q_{Alb} during the course of MS is poorly known, except for a regular increase due to aging. Higher values of Q_{Alb} are associated with poor outcome.^{18,19} Q_{Alb} remained unchanged after various drug treatments (steroids,²⁰ fingolimod,²¹ natalizumab²²), but decreased after pulsed high-dose steroids,^{23,24} natalizumab,^{25–27} and mitoxantrone.²⁸ In some cases, the IgG index may paradoxically increase as predicted in relation with a decreasing Q_{Alb} .²⁴ Therefore, quantification of ITS may often be related with Q_{Alb} and IgG_{Loc}(mean) should be used. Moreover, given the increasing dispersion of values as Q_{Alb} increases, the sensitivity of the traditional approximation decreases as long as Q_{Alb} increases. Therefore, while an ITS of 1 mg/L is easily detected with the lowest Q_{Alb} , a very large cohort is required to detect it when $Q_{\text{Alb}} = 10 (\times 10^{-3})$. On the other hand, we confirm that the use of formulas using Q_{Mean} in a cohort of patients provides a correct approximation of the exact IgG_{Loc}, although convergence below ± 2 mg/L is slow in higher Q_{Alb} .

Interestingly, this underestimation of intrathecal IgG synthesis is higher than the common range of many monoclonal antibody (mAb) concentrations required for biological activity, which are usually between 0.1 and 1 mg/L. Therefore, the amount of intrathecally synthesized IgG is sufficient to obtain a biological effect, even in the lower range of underestimated IgG synthesis. In other words, concentrations of intrathecally synthesized IgG may reach

the required lower limit for biological activity in the CSF long before any ITS is detected. Moreover, antibodies directed to extracellular targets and with predictable biological activity are retained in brain tissues.²⁹ Recent data suggest that autoreactive antibodies spilling over from the blood at a low level owing to an intact BCB may be completely cleared from the CSF by adsorption on CNS targets.^{30,31}

As a secondary outcome, we examined how to decipher ITS variations in two common clinical situations: plasma exchange (affecting serum IgG levels) and immune suppression targeting intrathecal IgG synthesis. Few data on this issue are available in the literature and the reliability of ITS measures obtained from patients after PLEX or immunosuppression is unknown.

We previously demonstrated that PLEX treatment reduces serum IgG levels and tunes CSF to an almost pure locally synthesized IgG level. The levels of antineuronal antibodies in serum and CSF before and after PLEX dropped only in serum, whereas CSF levels remained unchanged, but Q_{Alb} and Q_{IgG} were not reported.^{32,33} The IgG CSF/serum ratio increased after PLEX in most cases (anti-Yo and anti-Hu). In natalizumab-treated MS patients treated by PLEX in relation with a progressive multifocal encephalopathy (PML), IgG index increased³⁴ and the activity index (AI) against JCV increased 4-fold.³⁵ Although a rebound of local IgG synthesis induced by PML-immune reconstitution inflammatory syndrome (IRIS) could not be excluded, these results are in line with the predicted outcome of ITS after PLEX. However, in a single patient suffering from limbic encephalitis with ITS of multiple autoantibodies, variations in antibody indexes were heterogeneous and unpredictable in response to PLEX and add-on steroid treatments.³⁶ As a consequence, aside from the predictable biological effect of PLEX, one should keep in mind that ITS is highly dynamic in most clinical situations, obscuring the effect

of PLEX by a simultaneous unpredictable modification of ITS (i.e., increasing during an early ongoing inflammatory process and abating after steroid therapy).

Moreover, our results are based on the calculation of steady-state concentrations, which are supported by the dynamics of IgG (slow variations in blood, high CSF turnover). Delays to reach a new steady state after abrupt changes in BCB permeability or blood IgG concentrations are thought to be short but remain unknown. High doses of IgG (i.e., rituximab) injected in CSF are completely washed-out in less than 2 days.³⁷ Moreover, about half of the whole-body IgG is distributed in the interstitium (extravascular compartment) and the transcapillary escape rate (the transfer rate from the extra- to intravascular compartment) is about 3% per hour of the intravascular mass.³⁸ Therefore, the steady state of IgG levels in blood is obtained in approximately 2 days³⁹ and returns to pre-PLEX levels in a few weeks. Therefore, we consider that 2 days are sufficient to reach a steady state of IgG levels in blood and CSF. CSF drawn the day after PLEX still shows an IgG level suggestive of a former higher level of blood IgG, which may erroneously suggest the occurrence of ITS. In a study including 41 patients treated by PLEX, the IgG index was increased in 19% before PLEX, in 95% at day 1 post-PLEX, in 25% at day 2 and in 5% later.⁴⁰ Although 63% of these patients were suffering from Guillain-Barré syndrome (GBS), only a few of them also had multiple sclerosis, paraneoplastic cerebellitis or autoimmune encephalitis, which are all known to be associated with low level ITS. Considering our results, one cannot rule out that this apparently spurious ITS, especially in patients tapped at day 2 or later, may reveal a low level of true ITS. Madzar et al.⁴¹ provided CSF details from a single patient that allowed recalculation of Q_{IgG} . Their findings indicated a low level of ITS occurring in association with biases from nonsteady IgG concentrations. Future studies are required to decipher this hypothesis.

GBS is not usually considered to be associated with ITS.⁴² OCB are sometimes reported but are identical between blood and CSF (type 4, indicating passive transfer).⁴³ Considering the very high Q_{Alb} values observed in GBS (in the range of $20\text{--}80 \times 10^{-3}$), “silent ITS” could be more common than usually thought. Indeed, specific ITS was demonstrated for IgG against various antigens (α B-crystallin,⁴⁴ gangliosides,^{45,46} galactocerebrosidase⁴⁷), although there were some methodological concerns. For example, with $Q_{Alb} = 50(\times 10^{-3})$, if Q_{Mean} and $Q_{Lim}3SD$ are, respectively, 31.1 and 44.8, then Q_{IgG} is still below Q_{Lim} although a true ITS occurs up to $IgG_{Loc} = 100$ mg/L ($Q_{IgG} = 41.2$). In this situation, OCB would be blurred and a smaller ITS would be completely silent. Moreover, some of the control patients used to calculate Reiber’s formula were GBS patients, thereby introducing a potential

self-referencing bias. As a conclusion, the possibility of a small ITS associated with GBS should be reconsidered.

A major and unexpected result of Q_{Alb} variations is the paradoxical increase in IgG index and $IgG_{Loc}(Lim)$ in the absence of a real change in ITS. This pitfall is avoided by calculating $IgG_{Loc}(mean)$, which remains unchanged. However, it only concerns cases in which the level of ITS is low.

Since ITS may be a surrogate marker of a persistent intrathecal inflammation or be pathogenic by itself, any change in level may be a valuable target for future therapies.⁴⁸ Although slight changes may be difficult to demonstrate, we calculated that the size of the cohort required to demonstrate a decrease in ITS may be adjusted to the expected proportion of higher Q_{Alb} .

Conclusion

We herein demonstrate and quantify for the first time the range of underestimation of intrathecal IgG synthesis in individual patients. This range is higher than the lower common range of IgG concentrations required to obtain a biological effect. On the other hand, results obtained with cohorts fit well with the exact ITS value. Importantly, the sample size of a cohort needed to demonstrate slight variations in ITS requires adjustment with the expected BCB permeability.

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Author Contributions

BM conceptualized and designed the study; BM and GGM collected the clinical data. GGM and CH collected and managed the biological data. BM, BB, EK, MR, DH and DS clinically managed the data. BM, GGM, DH and DS analyzed and interpreted the data. BM, DH and DS performed the statistical analysis. BM drafted the manuscript; all authors critically evaluated the manuscript.

Conflict of Interest

Nothing to report.

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