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; \( Q_{\text{IgG}} \) increases after plasma exchange; \( \text{IgG}_{\text{loc}} \) calculation based on \( Q_{\text{lim}} \) falsely increases when \( Q_{\text{Alb}} \) decreases; the sample size required to demonstrate a decrease in ITS increases exponentially with larger \( Q_{\text{Alb}} \). Interpretation: Studies evaluating changes in ITS level should be adjusted to \( Q_{\text{Alb}} \). 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)\textsuperscript{3,4} and animal models of CNS autoimmunity.\textsuperscript{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), $Q_{\text{Alb}} = \frac{[\text{Alb}_{\text{CSF}}]}{[\text{Alb}_{\text{serum}}]}$, is a widely used parameter of BCB dysfunction that increases with its permeability and is influenced by age and underlying CNS pathologies. Normal maximal $Q_{\text{Alb}}$ is calculated by the formula: $(4 + \text{age(years)})/15 \times 10^{-3}$ 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 $[\text{IgG}_{\text{CSF}}]/[\text{IgG}_{\text{serum}}]$ is proportional to $Q_{\text{Alb}}$. Supposing a linear relation, Link et al. defined the IgG index $I_{\text{link}} = Q_{\text{IgG}}/Q_{\text{Alb}}$ with normal values $<0.7$.\textsuperscript{9} However, this linear cut-off did not precisely take into account either normal $Q_{\text{IgG}}$ variance or nonlinear correlation with $Q_{\text{Alb}}$ (ITS may be under- or over-estimated depending on low or high $Q_{\text{Alb}}$). Moreover, since $Q_{\text{Alb}}$ increases with age, the IgG index is thought to decrease mechanically without any change in ITS.\textsuperscript{9} In fact, data are best fitted by an empiric hyperbolic function (the “Reiber-gram”),\textsuperscript{10} whose constant parameters were later improved by a large dataset of 4154 control patients (supposed to be) devoid of ITS.\textsuperscript{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.\textsuperscript{11,12}

**Normal basal $Q_{\text{IgG}}$ ($Q_{\text{IgG\_basal}}$) 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 $Q_{\text{Alb}}$, in a population of normal patients, the distribution of $Q_{\text{IgG}}$ follows a normal law around the mean curve: $Q_{\text{mean}} = f(Q_{\text{Alb}})$ (Fig 1A). The variance of $Q_{\text{IgG}}$ defined by $[(Q_{\text{lim}} - Q_{\text{low}})/Q_{\text{mean}}]$ is about 0.91 for each $Q_{\text{Alb}}$.\textsuperscript{11} Individual variations in the diffusion pathway and CSF flow have been given as tentative explanations of this individual $Q_{\text{IgG}}$ variability.\textsuperscript{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:

$$Q_{\text{IgG}} = \frac{[\text{IgG}_{\text{CSF\_passive}}] + [\text{IgG}_{\text{CSF\_Loc}}]}{[\text{IgG}_{\text{serum}}]} = Q_{\text{IgG\_basal}} + Q_{\text{IgG\_Loc}};$$

(1)

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

The mean $Q_{\text{IgG}}$ among the normal population (therefore the mean $Q_{\text{IgG\_basal}}$) is defined by the $Q_{\text{mean}}$ curve. The upper limit of the reference range, $Q_{\text{lim}}$, is usually arbitrarily fixed as $Q_{\text{mean}} + 3SD$ and involves $\approx 99\%$ of the normal population. Using this definition, intrathecal IgG synthesis is acknowledged when $Q_{\text{IgG}} > Q_{\text{lim}}$. $Q_{\text{lim}}$ is commonly fixed at $Q_{\text{mean}} + 3SD$, since in the latter case only $<1\%$ of healthy controls may display a $Q_{\text{IgG}} > Q_{\text{lim}}$, giving a very high specificity to abnormal $Q_{\text{IgG}}$ values. A major drawback of this reference range is a loss of sensitivity, when cases displaying a low level of ITS ($Q_{\text{IgG}} > Q_{\text{IgG\_basal}}$ but $Q_{\text{IgG}} \leq Q_{\text{lim}}$), can be missed. In common practice, demonstration of ITS in these cases requires a CSF-restricted OCB positivity.

As expected, restriction of the reference range to $Q_{\text{lim}} + 2SD$ instead of $+3SD$, although increasing the risk of false positivity ($4\%$ of normal outside this range), increases the percentage of abnormal $Q_{\text{IgG}}$ in MS cohorts by 6–10% for IgG and up to 20% for IgM.\textsuperscript{9}

**Underestimation bias of local IgG synthesis—the “silent ITS”**

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

$$\text{IgG}_{\text{Loc}} = (Q_{\text{IgG}} - Q_{\text{IgG\_basal}}) \times [\text{IgG}_{\text{serum}}].$$

(2)

Since $Q_{\text{IgG\_basal}}$ is an unavailable parameter, a $Q_{\text{IgG\_norm}}$ replaces it, assuming $Q_{\text{IgG\_norm}} = Q_{\text{lim}}$ or $Q_{\text{mean}}$. As
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 $IgGLoc$ is variable and always underestimated, depending on the $Q_{IgG, basal}$ assumption, with the maximum of probability of $IgGLoc$ under the assumption of $Q_{IgG, basal} = Q_{mean}$. Underestimation of $IgGLoc$ is proportional to $Q_{Alb}$ (Fig. 1B, right panel). In the case of $Q_{IgG} = Q_{Lim} + 3SD$ ($\Delta Q_{IgG} = 0$), $IgGLoc$, based on $Q_{Lim}$ calculation, is estimated to be null in single individuals but

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**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 $IgGLoc$ should be calculated based on normal $Q_{IgG, basal}$ preceding disease onset (gray circle), which is unknown. In practice, $Q_{IgG,Loc}$ and therefore, $IgGLoc$, strongly depend on choice of possible $Q_{IgG, basal}$. By raising point B as a definitely abnormal $Q_{IgG} (>Q_{Lim})$, the range for the ratio of intrathecally synthesized IgG depends on estimation of basal (before disease onset) $Q_{IgG, basal}$. In individual patients, $Q_{mean} + 3SD$ (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 ($IgGLoc = (Q_{IgG} - Q_{IgG, basal}) \times [IgG_{serum}]$) is strongly influenced by arbitrary choice of $Q_{IgG, basal}$ (C) $IgGLoc$ depending on basal $Q_{IgG}$ estimation. $IgG_{Loc, Lim3SD} = 20$ mg/L, $IgG_{Loc, mean} = 43$ mg/L. Therefore, the true $IgG_{Loc, Simple}$ of $Q_{IgG}$ 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 $IgGLoc$ 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 $Q_{IgG, basal} = Q_{mean}$.
the underestimation falls within the range of 0 to 106 mg/L, in the \( Q_{\text{Alb}} \) interval 1 to 20 \( \times 10^{-3} \). With higher \( \Delta Q_{\text{IgG}} \), \( IgG_{\text{loc}} \) increases, whereas the absolute value of \( IgG_{\text{loc}} \) underestimation remains unchanged. As a consequence, this underestimated “silent” ITS can be closely approximated with a high level of probability by using \( Q_{\text{mean}} \).

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

\[
IgG_{\text{loc}} = (Q_{\text{IgG lokal}} - Q_{\text{IgG norm}}) \times [IgG_{\text{serum}}]. \tag{3}
\]

with \( IgG_{\text{loc}} \) is proportional to \( Q_{\text{IgG lokal}} \times (1-R) \), where \( R \) is the ratio of the \( Q_{\text{IgG norm}} \) and the true \( Q_{\text{IgG basal}} \) (i.e., \( R = \frac{Q_{\text{IgG basal}}}{Q_{\text{IgG basal}} \pm 1.5} \)). \( IgG_{\text{loc}} \) is null when \( Q_{\text{IgG basal}} \) is known (during simulation). On the other hand, the ratio of the \( IgG_{\text{loc}} \) variations remains negligible for the lowest values of \( R \), substantial amounts are concerned for higher values of \( R \). \( Q_{\text{mean}} \) increases along the \( Q_{\text{IgG basal}} \) axis. For \( Q_{\text{Alb}} \geq 4 \), the true \( Q_{\text{IgG}} \) is close to twice that of the calculated \( I_{\text{IgG}} \), and about thrice for \( Q_{\text{Alb}} \geq 7 \). The main consequence is that the fraction of “silent ITS” may be nonnegligible and even be major in the event of low \( IgG_{\text{loc}} \).

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 mechanistically increase since \( [IgG_{\text{serum}}] \) is the denominator of the fraction. However, for a concentration equilibrium, \( [IgG_{\text{CSF}}] = f ([IgG_{\text{serum}}]) \), meaning that both terms of \( Q_{\text{IgG}} \) 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. Therefore, \( Q_{\text{IgG}} \) is a constant for a given patient at a given BCB physiologic state, irrespective of \( [IgG_{\text{serum}}] \). As a counterintuitive consequence, although \( [IgG_{\text{serum}}] \) decreases after PLEX, both \( Q_{\text{IgG}} \) and IgG indexes remain constant in the absence of ITS:

\[
Q_{\text{IgG basal}} = \frac{[IgG_{\text{CSF passive}}]/[IgG_{\text{serum}}]}{[IgG_{\text{CSF}}]/[IgG_{\text{serum}}]}; \quad (5)
\]

so the predicted new level of CSF IgG is: \( [IgG_{\text{CSF}}] \) x \( [IgG_{\text{serum}}] \).

**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 (\( IgG_{\text{loc}} \)). In the equilibrium state (before lowering \( [IgG_{\text{serum}}] \)):

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

\[
Q_{\text{IgG}} = \frac{[IgG_{\text{CSF passive}} + IgG_{\text{loc}}]/[IgG_{\text{serum}}]};
\]

\[
Q_{\text{IgG}} = \frac{Q_{\text{IgG basal}} + IgG_{\text{loc}}}{[IgG_{\text{CSF passive}}]/[IgG_{\text{serum}}] + IgG_{\text{loc}}/[IgG_{\text{serum}}]};
\]

Therefore, after lowering the initial \( [IgG_{\text{serum}}] \) to a lower \( [IgG_{\text{serum}}] \):

\[
Q_{\text{IgG}} = \frac{[IgG_{\text{CSF passive}}]/[IgG_{\text{serum}}] + IgG_{\text{loc}}/[IgG_{\text{serum}}]}{[IgG_{\text{CSF passive}}]/[IgG_{\text{serum}}];
\]

\[
Q_{\text{IgG}} = \frac{Q_{\text{IgG basal}} + IgG_{\text{loc}}}{[IgG_{\text{loc}}]/[IgG_{\text{serum}}]};
\]

As a consequence, \( Q_{\text{IgG}} \) decreases proportionally to the right term of the equation 6, \( Q_{\text{IgG loc}} \). 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
PLEX, poor physiological condition or chronic infection do not modify $Q_{\text{Alb}}$.\textsuperscript{12,13}

**Closing the BCB increases $Q_{\text{IgG}}$ 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 $Q_{\text{Alb}}$ and $Q_{\text{IgG}}$. 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 $Q_{\text{IgG}}$ (Eq. 1), where only $Q_{\text{IgG, basal}}$ decreases whereas $Q_{\text{IgG, Loc}}$ remains constant. This change tunes the contrast of ITS among the IgG CSF concentrations and may shift apparently normal $Q_{\text{IgG}}$ 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
from $Q_{\text{Alb}}(\times 10^{-3}) = 8$ 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 IgGLoc, which is based on $Q_{\text{Lim}}(3SD)$ for single patient calculation, may incorrectly confirm this apparent onset of ITS, whereas calculation based on $Q_{\text{mean}}$ 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 $Q_{\text{IgG},\text{basal}}$ is known. Therefore, using a simple mathematical model based on $Q_{\text{Lim}}(3SD)$ for single patient calculation, may incorrectly confirm this apparent onset of ITS, whereas calculation based on $Q_{\text{mean}}$ is accurate.

**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 $Q_{\text{IgG}}$ and IgGLoc 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 $Q_{\text{Alb}}$ (Fig. 5A). AUC increased along with ITS but was inversely proportional to $Q_{\text{Alb}}$. Low amounts of ITS were poorly discriminated at high $Q_{\text{Alb}}$. At single patient level,
estimation of true IgGLoc was poor and IgGLoc(Lim) was highly unreliable (Fig. 5B). We compared true IgGLoc values with the results obtained at a cohort level using the various possible calculations (not shown). A correct approximation of the true IgGLoc was systematically obtained for calculations based on $Q_{\text{Mean}}$, even with IgGLoc levels lower than 1 mg/L. On the other hand, estimations of IgGLoc based on $Q_{\text{Lim}}$ at 2SD or 3SD gave arbitrary results. Moreover, the precision of the results based on $Q_{\text{Mean}}$ was independent from $Q_{\text{Alb}}$, whereas estimation based on $Q_{\text{Lim}}$ was strongly biased (results not shown). However, calculation of IgGLoc(mean) based on the simulation of small cohorts of patients demonstrated that the speed of convergence of IgGLoc was independent from the true ITS and inversely proportional to $Q_{\text{Alb}}$ (Fig. 5C). The proportion of abnormal $Q_{\text{IgG}}$ strongly depended on the ITS level and the $Q_{\text{Alb}}$ (Fig. 6). For a null ITS (normal state), about half of the $Q_{\text{IgG}}$ were situated above $Q_{\text{Mean}}$ whereas only 13% were higher than $Q_{\text{Lim}} + 3\text{SD}$ (with $Q_{\text{Alb}} = 4 \times 10^{-3}$). As a consequence, the population bias $Q_{\text{IgG}} > Q_{\text{Mean}}$ 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 $Q_{\text{IgG}}$ with an unexpected sensitivity. An increase in IgG index is even predicted for extremely low levels of ITS (1 mg/L) when [IgGserum] is decreased by more than 90%. For an ITS value of 10 mg/L ($Q_{\text{IgG}}$ remains under $Q_{\text{Lim}}$), a minimal decrease in [IgGserum] of 20% is sufficient to turn the IgG index and $Q_{\text{IgG}}$ into abnormal values. Results obtained for common ITS values and PLEX outcome are listed in Table 1. Note that when [IgGserum] was lowered even more, the fraction of IgGCSF in CSF became substantial even at very low ITS rates (i.e., up to 30% of IgGCSF is of local origin for ITS = 1 mg/L and PLEX rate = 90%). Interestingly, after a PLEX procedure deleting [IgGserum] by 90%, [IgGCSF] became almost a pure product of locally synthesized IgG.

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

| Distributions of observed variables | Random generation | Simulated population |
|-------------------------------------|-------------------|----------------------|

- $Q_{\text{Alb}}$ [0-10] (x10-3) → $Q_{\text{Alb}}$ (n=4600) Random association
- QlgG of healthy controls $Q_{\text{mean}} \pm 3\text{SD} [Q_{\text{Lim}}-Q_{\text{Low}}]$ → Normal QlgG → QlgG_basal Random association
- Serum [IgG] in healthy controls → [IgG serum] → Normal serum [IgG] → Simulated normal population (n=4600)
- Tuneable constant ITS (mg/L) → Intrathecal IgG synthesis (SIT) → Simulated constant ITS ‘C-ITS cohort’ (n=4600) → Reibergram (fig. 7)
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 $Q_{IgG}$. 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 IgGLoc remains unreliable, especially based on $Q_{Lim}$. (C) Convergence of the error range (in absolute value) of IgGLoc 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: $|\text{mean}(\text{IgGLoc\_true}) - \text{mean}(\text{IgGLoc\_calculated})|$. For each condition ($Q_{Alb}$, ITS), up to 60 subjects are included in 12 independent simulated assays.
Simulations of PLEX in the C-ITS cohort are depicted in Figure 7. PLEX increased 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 [IgGserum] by 80%. Note that the precision of the IgGLoc 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 IgGLoc (mean) was stable whatever the fixed variations of the ITS level, but it increased collinearly with QAlb levels (1.7–8.7 in the QAlb interval 2–10 × 10⁻³). Therefore, the sample size depends on the distribution of QAlb in the tested population. Sample size estimations are provided for a range of ITS variations depending on QAlb. 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., 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 Ig synthesis in up to 21–39% of MS cases and in up to 90% of paraneoplastic disorders. 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 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 QIgG to QAlb is a nonlinear curve. While methods for quantifying ITS (IgGLoc) have been largely debated and several formulas have been proposed, the formula derived from the Reibergram remains the best approach although it provides only an approximation. However, as we demonstrate above, whatever the choice of QLim cut-off, results concerning “silent ITS” remain mostly unchanged: that is, ROC curves calculated (not shown) with the procedure described by Auer et al. are very close to those obtained with Reiber’s formula.
“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 1. Main CSF parameters after lowering serum IgG level by plasma exchange (PLEX).

| [IgGserum] (mg/L) | True ITS ($Q_{IgG}$) (mg/L) | IgG index | $Q_{IgG} (\times 10^{-3})$ | $Q_{IgG loc (Lim)}$ (mg/L) | $Q_{IgG loc}$ (mg/L) | True $IF_{IgG}$ (%) | Estimated $IF_{IgG}$ (%) |
|------------------|----------------------------|-----------|--------------------------|---------------------------|---------------------|-------------------|----------------------|
| **Before PLEX**  |                            |           |                          |                           |                     |                   |                      |
| 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). $Q_{IgG loc} = Q_{mean}$ is used for the sake of clarity. Starting parameters: $Q_{Alb} = 5 \times 10^{-3}$; $Q_{IgG_{basal}} = 2.3 \times 10^{-3}$; $Q_{Alb(3SD)} = 3.47 \times 10^{-3}$; $[IgGserum] = 10$ 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 $[IgGserum]$ is reduced by 50%. Estimated $IF_{IgG}$ increases from 0% to 52% for a 90% decrease in basal $[IgGserum]$.

### 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 $\times 3.6$).
ITS may give the misleading impression of changes in ITS. For example, if \( Q_{\text{Ab}} \) 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 \( Q_{\text{Ab}} \) decreases. In this case, the calculation of \( \text{IgG}_{\text{Loc}} \) should not be based on \( Q_{\text{Lim}} \) but rather on \( Q_{\text{mean}} \) which minimizes the risk of error.

\( \text{IgG}_{\text{Loc}} \) in MS patients is sometimes slightly higher than expected with age, but evolution of \( Q_{\text{Ab}} \) during the course of MS is poorly known, except for a regular increase due to aging. Higher values of \( Q_{\text{Ab}} \) are associated with poor outcome.\(^{18,19} \) \( Q_{\text{Ab}} \) remained unchanged after various drug treatments (steroids,\(^{20} \) fingolimod,\(^{21} \) natalizumab\(^{22} \)), 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 \( Q_{\text{Ab}}. \)\(^{24} \) Therefore, quantification of ITS may often be related with \( Q_{\text{Ab}} \) and \( \text{IgG}_{\text{Loc}} \) (mean) should be used. Moreover, given the increasing dispersion of values as \( Q_{\text{Ab}} \) increases, the sensitivity of the traditional approximation decreases as long as \( Q_{\text{Ab}} \) increases. Therefore, while an ITS of 1 mg/L is easily detected with the lowest \( Q_{\text{Ab}}, \) a very large cohort is required to detect it when \( Q_{\text{Ab}} = 10(\times 10^{-3}) \). On the other hand, we confirm that the use of formulas using \( Q_{\text{mean}} \) in a cohort of patients provides a correct approximation of the exact \( \text{IgG}_{\text{Loc}} \), although convergence below \( \pm 2 \) mg/L is slow in higher \( Q_{\text{Ab}} \).

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 \( Q_{\text{Ab}} \) and \( Q_{\text{IgG}} \) 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 increased\(^{34} \) 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

### Table 2. Sample size estimation according to ITS changes and \( Q_{\text{Ab}} \) strata.

| \( Q_{\text{Ab}} (\times 10^{-3}) \) | 2    | 4    | 6    | 10   |
|----------------------------------|------|------|------|------|
| \( \text{IgG}_{\text{Loc}} \) SD (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   |
| \( \text{IgG index (SD)} \) Basal | 0.42 (0.08) | 0.45 (0.08) | 0.48 (0.08) | 0.52 (0.08) |
| \( +\text{ITS} \) 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 \( P = 0.05. \)

\( ^{1} \)SD of \( \text{IgG}_{\text{Loc}} \) are similar whatever the level of basal ITS.
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. 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. Therefore, the steady state of IgG levels in blood is obtained in approximately 2 days 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. Although 63% of these patients were suffering from Guillaumin–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. provided CSF details from a single patient that allowed recalculation of QIgG. 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. OCB are sometimes reported but are identical between blood and CSF (type 4, indicating passive transfer). Considering the very high QAlb values observed in GBS (in the range of 20–80 × 10–3), “silent ITS” could be more common than usually thought. Indeed, specific ITS was demonstrated for IgG against various antigens (zB-crystallin, gangliosides, galactocerebrosidase), although there were some methodological concerns. For example, with QAlb = 50(×10–3), if Qmean and Qlim3SD are, respectively, 31.1 and 44.8, then QIgG is still below Qlim although a true ITS occurs up to IgGLoc = 100 mg/L (QIgG = 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 QAlb variations is the paradoxical increase in IgG index and IgGLoc(Lim) in the absence of a real change in ITS. This pitfall is avoided by calculating IgGLoc(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. 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 QAlb.

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, EH, MR, DH and DS clinically managed the data. BM, GGM, DH and DS analyzed and interpreted the data. BM, BB, EH, MR, 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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