Introduction
to CSF Diagnostics

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Your Partner in Infectious Serology

Cerebrospinal fluid and CSF diagnostics

The cerebrospinal fluid (CSF) surrounds the brain and the spinal cord. Its primary function is physical protection of the central nervous system (CNS). The fluid spaces surround the ventricle in the brain and the subarachnoid space (inner and outer fluid spaces). Approximately 80 to 90 % of the cerebrospinal fluid is formed in the choroid plexus from the blood. Every day 500 ml of fluid are produced, which is about three times the volume of fluid of 150 ml in an average adult.

Most of the cerebrospinal fluid is drained into the venous blood via the arachnoid villi. The process of formation is triggered by the difference in pressure between arterial and venous blood. Blood and cerebrospinal fluid are physiologically separated by the blood-CSF-barrier (Fig. 1). This barrier is permeable for specific soluble substances, with the permeability being dependent on, among other factors, the size of the molecules. An intact blood-CSF-barrier significantly reduces the concentration of protein in the cerebrospinal fluid. For example, the concentration of albumin in the fluid is approx. 200 times less than in the serum. Most of the proteins in the CSF have entered the fluid space from the serum via the blood-CSF-barrier.

CSF diagnostics is an important part of the diagnostic process for the detection of neurological diseases. Examination of the cerebrospinal fluid can be used to confirm or exclude the presence of acute and chronic inflammatory reactions involving the CNS, autoimmune diseases (e. g. multiple sclerosis), subarachnoid bleeding, neoplasms with infiltration to the CNS and neurodegenerative diseases. The objective of CNS diagnostics is to prepare a comprehensive, patient-oriented report that can present all measured parameters. The recorded data can be analyzed for plausibility and interpreted on the basis of the report. The detection of patterns related to disease requires a large number of high-quality diagnostic analyses, which can be classified into different stages.

As part of an emergency program a visual examination is performed, cell type and numbers, lactate and protein content are determined. If there is a suspicion, the basic program includes incubation of bacteria, an analysis of the function of the blood-CSF-barrier, detection of an intrathecal antibody synthesis, and the determination of oligoclonal bands in CSF and serum. Depending on the results of the above tests, a special analysis program can be run to confirm the presence of pathogen-specific antibodies in the fluid and serum, which depending on the differential diagnostic question or molecular biology procedures can also be included.

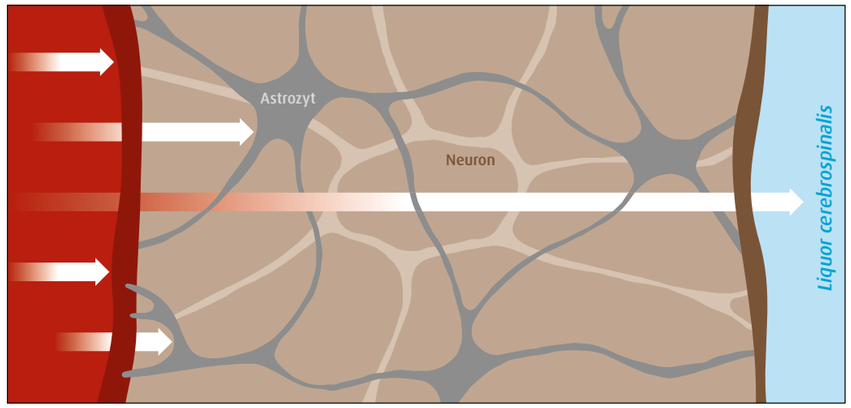

Fig. 1:
Physiological view of the blood-CSF-barrier

Analyses in CSF diagnostics

Visual assessment
Cerebrospinal fluid is colorless, clear and contains very few cells (< 4/µl). The macroscopic assessment classifies it into cloudy (> 1000 cells/µl), or purulent (> 10,000 cells/µl) which is caused by an increase in cell count. Bloody or xanthochromic CSF may be induced by artificial or subarachnoid bleedings.

Cell count and differentiation
In the absence of a pathological condition the cerebrospinal fluid contains fewer than 4 cells/µl, which are lymphocytes or monocytes. The presence of plasma cells, activated B-lymphocytes, and macrophages indicates an inflammatory reaction. Inflammatory reactions involving the central nervous system generally result in an increase in the cell count (pleocytosis).

Glucose and lactate
The glucose content in the fluid depends on the content in the serum. The reliability of the analysis is somewhat restricted because the serum glucose content varies and the transfer of variations to the fluid is delayed. The concentration of lactate in the cerebrospinal fluid is independent of the content in the serum and must therefore be measured in the fluid only. Inflammatory reactions involving the central nervous system due to bacteria or fungi, in contrast to viral processes, generally result in an increase in the lactate concentration in the fluid.

Blood-CSF-barrier
The functioning of the blood-CSF-barrier is strongly influenced by the speed of the CSF flow. A reduced fluid flow causes a blood-CSF-barrier-dysfunction. A reduction can be caused by a reduced formation or blockage of drainage. Because albumin is only synthesized in the liver, all molecules detected in the cerebrospinal fluid must have passed through the blood-CSF-barrier. As a result, the albumin quotient Q_{Alb} can be used as a measure of the functioning of the blood-CSF barrier.

$$Q_{Alb} = \frac{[Alb_{CSF}]}{[Alb_{Serum}]}$$

If an increased albumin content in relation to the reference value in the serum is detected in the cerebrospinal fluid, this is an indicator of dysfunction of the barrier. The albumin quotient reaches its minimum at the age of four months to five years and then continuously increases due to the age-related decline in production of cerebrospinal fluid.

Intrathecal antibody synthesis
The blood-CSF-barrier, which physically separates blood and cerebrospinal fluid from each other, is permeable for specific soluble substances. The different sizes of immunoglobulins results in different diffusion rates into the fluid space (IgM < IgA < IgG). As a result of the diffusion of immunoglobulins from serum into the cerebrospinal fluid, the intrathecal antibody synthesis must always be separated from antibodies of serological origin. Thus increased antibody activity in the serum must increase the concentration of antibodies in the CSF, even in the absence of an intrathecal antibody synthesis. Dysfunction of the blood-CSF-barrier also changes the antibody activity in the cerebrospinal fluid. As a result, in the detection of an intrathecal antibody synthesis both the cerebrospinal fluid and the serum must be analyzed and the ratio of each determined.

The intrathecal immune reaction depends on the infection pathogen. In contrast to serological analyses, a change in the antibody class from IgM to IgG is not common, because there are no corresponding regulatory structures. As a result, intrathecally synthesized IgM or IgA antibodies can be confirmed even with chronic or past infections with CNS involvement. The synthesis of single classes of immunoglobulins dominates in the case of specific manifestations. The analysis of the relative proportions of intrathecal IgG, IgA and IgM fractions forms the basis of disease-related antibody reaction patterns. Antibodies of the IgM class are dominant in neuroborreliosis, while an intrathecal antibody synthesis is commonly detected with neurotuberculosis. The MRZ(H) reaction is used to confirm a multiple sclerosis. The combined intrathecal confirmation of single or multiple IgG antibody activities is used as the marker for this reaction (see p. 8, section Interpreting results).

Oligoclonal bands
Oligoclonal bands are confirmed using isoelectric focusing (IEF). The technique is a sensitive method of confirming intrathecal IgG antibody production. This high-resolution electrophoresis separates immunoglobulins based on their isoelectric points. Then the patterns of the bands in the serum and fluid samples are compared. In an intrathecal antibody synthesis bands can be detected in CSF samples that cannot be detected in the serum.

Reiber quotient graphs
The Reiber quotient graph (also referred as the Reiber diagram) is used to determine intrathecal antibody syntheses of immunoglobulins of the IgA, IgG or IgM classes. The quotient of the antibody activity (Q_{IgA} Q_{IgG} Q_{IgM}) in the cerebrospinal fluid and serum is blotted in the graphs against the albumin quotients (Q_{Alb}). The graphs are based on the examinations of healthy subjects.

The standard range of albumin quotients depends on age and is marked by a vertical line (Fig. 2, a). If the limit value is exceeded, i. e. there is an increased albumin content in the cerebrospinal fluid compared to the concentration in the serum, there is a barrier dysfunction. Because as with increasing permeability of the blood-CSF-barrier the content of immunoglobulin in the CSF will also be rising, the normal ranges for antibody quotients increase with enhanced albumin quotients.

In the presence of an intrathecal antibody synthesis the antibody activity in the cerebrospinal fluid increases compared to the activity in the serum. As a result, an increased immunoglobulin quotient is detected. In this case the Q_{IgX} exceeds the $Q_{Lim\ IgX}$ which corresponds to the upper normal range of healthy subjects depending on the albumin quotients. The Reiber graphs show normal findings (Fig. 2, 1), dysfunctions of the blood-CSF-barrier (Fig. 2, 2), intrathecal antibody syntheses (Fig. 2, 4), and also combined dysfunctions of the blood-CSF-barrier and antibody syntheses (Fig. 2, 3). If there is an intrathecal synthesis, the percentage of antibodies formed in the cerebrospinal fluid compared to the total concentration can be estimated. The lines above $Q_{Lim\ IgX}$ can be used for the estimate (Fig. 2, b).

Determination of the antibody index
The pathogen-specific antibody index (AI) indicates the antigen-specific IgG, IgM or IgA concentration synthesized in CSF. Similar to the determination of the total antibody content by oligoclonal bands or with Reiber graphs, specific antibody activity in the cerebrospinal fluid must also be compared with the content in the serum in order to distinguish an intrathecal antibody synthesis from antibodies of serological origin. For this reason the specific antibody quotient $Q_{spec.\ IgX}$ is calculated:

$$Q_{spez.\ IgX} = \frac{[spez.\ IgX_{CSF}]}{[spez.\ IgX_{Serum}]}$$

A dysfunction of the blood-CSF-barrier may cause increased antibody activity in the cerebrospinal fluid and thus to an increase of the $Q_{spec.\ IgX}$. The $Q_{spec.\ IgX}$ must referred as a marker for the barrier function to preclude a misinterpretation. To simplify the method, the previously described albumin quotient Q_{Alb} is not used. Instead, the IgX quotient Q_{IgX} derived from the IgX concentration in the cerebrospinal fluid and the content in the serum is used as the basis for the blood-CSF-barrier function. In healthy individuals the CSFserum ratio of specific IgX is identical to the ratio of the total IgX antibodies. For this reason Q_{IgX} can be used as a reference and replaces the reference to the albumin quotient Q_{Alb} as a barrier parameter. The following calculation method is to derive the antibody index:

$$AI = \frac{[Q_{spez.\ IgG}]}{[Q_{IgG\ total}]}$$

Because the ratio of the specific antibody quotient $Q_{spec.\ IgX}$ in the absence of a pathological conditions corresponds to the quotient of all antibodies of that class, a value of AI = 1 would be expected in healthy individuals. The specific antibody activity in CSF will increase in the case of intrathecal syntheses. This will lead to increased $Q_{spec.\ IgX}$ values. As initially the intrathecal antibody synthesis will only slightly enhance the total antibody activity in CSF the Q_{IgX} will remain almost unchanged. As a consequence the AI will increase and can serve as a marker for intrathecal antibody syntheses. Due to potential inaccuracies and variations in the determination of the analytes, antibody indices between AI = 0.7–1.4 are normally interpreted as standard

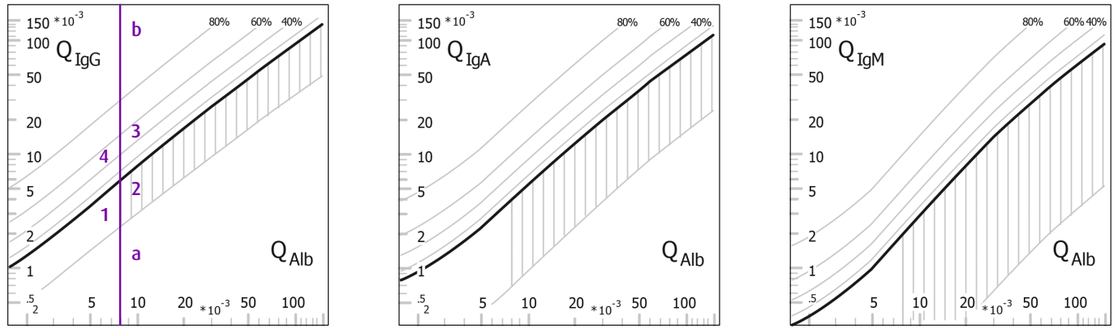

Fig. 2: Reiber graphs of a 60-year-old patient

findings while AI > 1.5 is usually evaluated as pathological.

As noted, the AI value calculation of Q_{IgX} can be used as a measure of the blood-CSF-barrier instead of Q_{Alb}. However, a strong or polyspecific stimulation of B-lymphocytes in the fluid space may occur in the presence of a pathological condition. An extensive specific intrathecal antibody sythesis will increase the total antibody activity in the cerebrospinal fluid strongly. As a result Q_{IgX} significantly increases and loses its significance as a marker for the blood-CSF-barrier, because a distinction between diffusion and intrathecal synthesis is no longer possible. However, if the increased Q_{IgX} is used for calculation, the antibody index will be reduced, which may lead to incorrect non-pathological results. For this reason, the AI calculation with a strong or polyspecific stimulation is not based on the Q_{IgX}. The upper normal range $Q_{Lim\ IgX}$ is used instead, which represents the maximum Q_{IgX} occurring in healthy persons. The use of $Q_{Lim\ IgX}$ eliminates the possibility of an intrathecal synthesis falsifying the marker of the blood-CSF-barrier function. This effect can be ignored in the absence of a strong or polyspecific stimulation. As a result, two cases must be distinguished when calculating the antibody index (AI):

$$\text{a) } AI = \frac{[Q_{spez.\ IgG}]}{[Q_{IgG\ total}]} \qquad \text{b) } AI = \frac{[Q_{spez.\ IgG}]}{[Q_{Lim\ IgX}]}$$

DGLN recommendations for calculating the antibody index

The recommendations of the German Society for CSF Diagnostics and Clinical Neurochemistry (DGLN) are the most comprehensive specifications for quality assurance in CSF diagnostics. The DGLN recommendations for calculating the antibody index are described below.

1. Quantitative determination of antibody activity in CSF and serum

CSF diagnostics requires accurate determination of antibody activities. Therefore, analytical procedures for calculating the AI that are not based on concentrations are not recommended. Calculation of an antibody index based on OD (optical density) suggests a linear relationship between measured values and antibody activities. OD values do not have a linear relationship with the antibody activities. Thus, if measured values of CSF and serum are not identical, discrepancies from the actual activities are included in the AI analysis.

2. Analysis of the CSF and serum sample in one test run

If the CSF and serum samples are analyzed in different test runs, variations in the test level between the different preparations may falsify the AI calculation.

3. Dilutions

Matrix effects must be eliminated to ensure an accurate quantification of antibodies. They are induced by interferring substances but not by the analyte. They are elicited by divalent ions and compounds of non-specific components. At low dilutions the matrix of the test material is more concentrated. To prevent matrix effects CSF must be diluted before analysis. Because the cerebrospinal fluid is a filtrate of serum, the antibody activities in these samples are commonly low. Therefore, excessively high dilutions should not be selected. In general, CSF is initially analyzed at a dilution of 1:2.

Because the quantification of the antibodies does not show a linear relationship between measured signal and analyte, the experimental standard curve must be correlated with a mathematical curve function. In addition, the immune system synthesizes a wide range of antibodies to various epitopes of a pathogen, which vary in concentration, specificity and binding strength, such as affinity and avidity. As a result, systematic errors occur that may influence the result when analyzing the antibodies. Because the systematic errors vary in ranges of different antibody activities, CSF and serum samples with similar activity should be used to determine the antibody index. Systematic error is generally low in the pseudo-linear range of the quantification curve, which means that the difference between the antibody activities for CSF and serum do not absolutely need to be very low. In contrast to this, outside the pseudo-linear range only similar antibody activities are used to determine the AI.

The initial serum dilution should be at least 1:400, because antibodies in the CSF are generally significantly less concentrated. In the ideal case, dilutions are selected so both activities are in similar ranges on the standard curve.

A very accurate analysis over a wide range is possible within the pseudolinear section. The activities should be less strongly differentiated close to the quantification limits. Due to this dependency on the acceptable antibody response, it is not possible to formulate a general rule on the allowable maximum difference between the two samples. The sample should be analyzed for dilution linearity to determine the exact AI. It may be affected by matrix or displacement effects. For this reason, in most cases different dilutions of the CSF and serum material are tested. Antibody index analyses of samples that are not dilution-linear are not reliable. Samples often behave in a linear way only at specific dilutions. The AI calculation must be performed only with the measured values from this range.

4. Measurement range limits and borderline ranges

Quantification limits of immunoassays are generally defined by the precision (reproducibility) of measured values. In sections far outside the pseudolinear range of the standard curve minor deviations of the measured values lead to major deviations of the activity evaluation. These sections are not included in the measurement range. This means that an AI analysis should only be performed with antibody activities that are inside the measurement range. In addition, values OD < 0.1 are not used, because they are generally below the technical cut-off point. This precludes reliable quantification. The commonly described restriction to OD < 2.0 values must be viewed historically and was due to the restricted measurement ranges of previous photometers.

Serological borderline ranges are not relevant in CSF diagnostics. They are determined in clinical trials by analyzing serum samples from patients suspected of having an infection and from healthy blood donors and are based on the dilution for serological analyses. The cut-off that must be used in CSF diagnostics is AI = 1.5. As described it is calculated as a ratio from the antibody activity in CSF and serum. With the ratio pathological AIs can also be detected using antibody activities within or outside the range of the borderline range.

Interpretation of results

The results of the analyses of CSF diagnostics should be interpreted with the inclusion of as many parameters as possible. After interpretation of the individual measured values to check the plausibility of the recorded data, the results can be used to identify disease-related patterns. It is important at this point to combine the results of different analyses; findings can generally not reliably interpreted based on the evaluation of the results of single analyses. The significance of considering different results is to be explained with the following disease patterns that commonly occur in CSF diagnostics.

In the case of acute viral or bacterial infections involving the CNS (e. g. neuroborreliosis, encephalitis or viral meningitis, etc.), the initial symptom is generally activation of monocytes and specific B-lymphocytes. The activation induces the proliferation of cells, which leads to detection of increased cell count in the cerebrospinal fluid (pleocytosis). A strong pleocytosis can lead to cloudy or purulent apperance of CSF. In the case of bacterial infections, the lactate concentration increases over an extended period while the level of CNS lactate with viral infections is increased only in the early phase. In the course of the disease the blood-CSF-barrier is generally affected with bacterial infections and only partially with viral infections, which among other results leads to a rise in the albumin concentration in the CSF. This means that a barrier dysfunction can be detected based on an increased albumin quotient Q_{Alb}.

The specific stimulation of the B-lymphocytes accompanying the infection causes intrathecal synthesis of antibodies, which are directed against the triggering pathogens. This causes a rise in the specific antibody activity and the total concentration of antibodies of specific classes of immunoglobulins in the CSF and thus to increased $Q_{spec.IgX}$ and Q_{IgX}. Correspondingly, intrathecal antibody syntheses can be detected using the Reiber graphs. Intrathecal syntheses of antibodies of the IgG class can also be confirmed by oligoclonal bands using isoelectric focusing. Increased antibody indices in pathological ranges are also found, which can be calculated based on the specific antibody activities.

Synthesis of antibodies of various classes of immunoglobulins may also be found with the described acute infections. For example, in the case of neuroborreliosis and intrathecal syntheses of antibodies the IgM and IgG classes are isolated or detected in combination. The analysis of additional classes of immunoglobulins may also be useful in addition to the confirmation of IgG antibodies with virus infections involving the CNS. In general, note that particularly with diseases originating from herpes viruses very high antibody activities may be encountered, which is why it is difficult to select suitable dilutions. Because the B-lymphocytes in the CSF are not fully regulated, increased antibody indices of various classes of immunoglobulins may also be detected after convalescence. Note that a rise in the AI may be observed that is without clinical relevance due to decreasing antibody indices in the serum with stable activity in the CSF. In general, it can be assumed that there may be a diagnostic gap between the time of infection and the ability to confirm the antibody synthesis.

The confirmation of a multiple sclerosis (MS) may also be due to the detection of intrathecal syntheses of pathogen-specific antibodies. Infections of specific pathogens can certainly not be connected with the occurrence of multiple sclerosis. However, B-lymphocytes are activated as a result of non-specific activation of the immune system as part of MS, which in approx. 90 % of patients leads to an intrathecal antibody synthesis. What is referred to as the MRZH reaction has become established as a marker for multiple sclerosis. It consists of the combined confirmation of an intrathecal synthesis of antibodies of the IgG class against measles virus (“M”; in approx. 80 % of patients), rubella virus (“R”, in approx. 65 % of patients), varicella virus (“Z”, in approx. 50 % of patients) and herpes simplex virus (“H”, in approx. 25 % of patients). If increased antibody indices can be confirmed with one or more parameters, this is an initial indication of multiple sclerosis. However, it must be noted that normally increased cell counts or lactate concentrations cannot be detected, because there is no acute infection. This also means that diagnosis by PCR is not possible. In contrast to many acute infections involving the CNS, the blood-CSF barrier function is not affected with MS.

CSF Report

Patient: Information: Date of birth: Sample received:

Differential diagnostic query:	Date of puncture:

Puncture	Visual assessment	Hgb.	Vol.	TP (Pandy)
LP CP VP	turbid xanth. bloody art. bl.	0 + ++ +++	ml	0 + ++

Cells

Cellcount /mm³		RBC /mm³	
Lymphoc. %	Mononuc. C. %	PMNR (n.) %	Plasmacells %

Other cells	
Activated B-Cells	% of Lymphocytes

Proteins

	CSF	Serum	Q (CSF/Ser) X 10³	Intrathec. fraction
Tot.	mg/L			
Alb.	mg/L	g/L	Q_{Alb}	
IgG	mg/L	g/L	Q_{IgG}	%
IgA	mg/L	g/L	Q_{IgA}	%
IgM	mg/L	g/L	Q_{IgM}	%

Oligoclonal IgG

CSF	Serum

Specific Antibodies

Measles V. AI	HIV AI	Borrel. IgG AI
Rubella V. AI	CMV AI	Borrel. IgM AI
VZV AI	Toxopl. AI	..... AI
HSV AI	..... AI	..... AI

Lactate

mmol/

Comments

CSF diagnostics with SERION ELISA

To calculate the antibody index, pathogen-specific immunoglobulins must be detected in serum and CSF. Institut Virion\Serion GmbH offers a wide range of CE-certified assays that are validated for antibody detection in CSF diagnostics. An analysis for CSF diagnostics can be performed with a conventional SERION ELISA identical to serological analyses; expensive additional reagents are not required. The test systems enable determination of CSF and serum samples at different dilutions in one test preparation. The user can select different dilutions of the cerebrospinal fluid and different dilutions of the serum and refer to the same set of calibrators. Determination of the antibody activities of the serum analyses of the CSF diagnostics can also be used for serology by taking the dilution factor into account. The use of unified incubation periods (including for CSF diagnostics and serology) enables different SERION ELISA to be combined in one test run. Antibody activities can be determined precisely and over a wide measurement range due to the accurate quantification with the 4 PL method used for the SERION ELISA. The single-point calibration to a preset, lot-specific standard curve enable economical processing without expensive and time-intensive adjustment of standard curves based on a high number of calibrators.

Highlights of the SERION ELISA Immunoassays

- Comprehensive product range for CSF diagnostics
- Detection of intrathecal synthesized antibodies using the method developed by Prof. Hansotto Reiber
- Calculation of antibody indices with the software SERION *easy*CSF
- Identical test systems for serology and CSF diagnostics; additional expensive reagents are not required
- Parallel processing of CSF diagnostics and serology
- Exact quantification of antibody activities by use of the precise 4-parameter logistics function (4-PL)
- Different dilutions of serum and CSF samples can be selected and combined at will to calculate the antibody index
- Automated CSF diagnostics with SERION Immunomat

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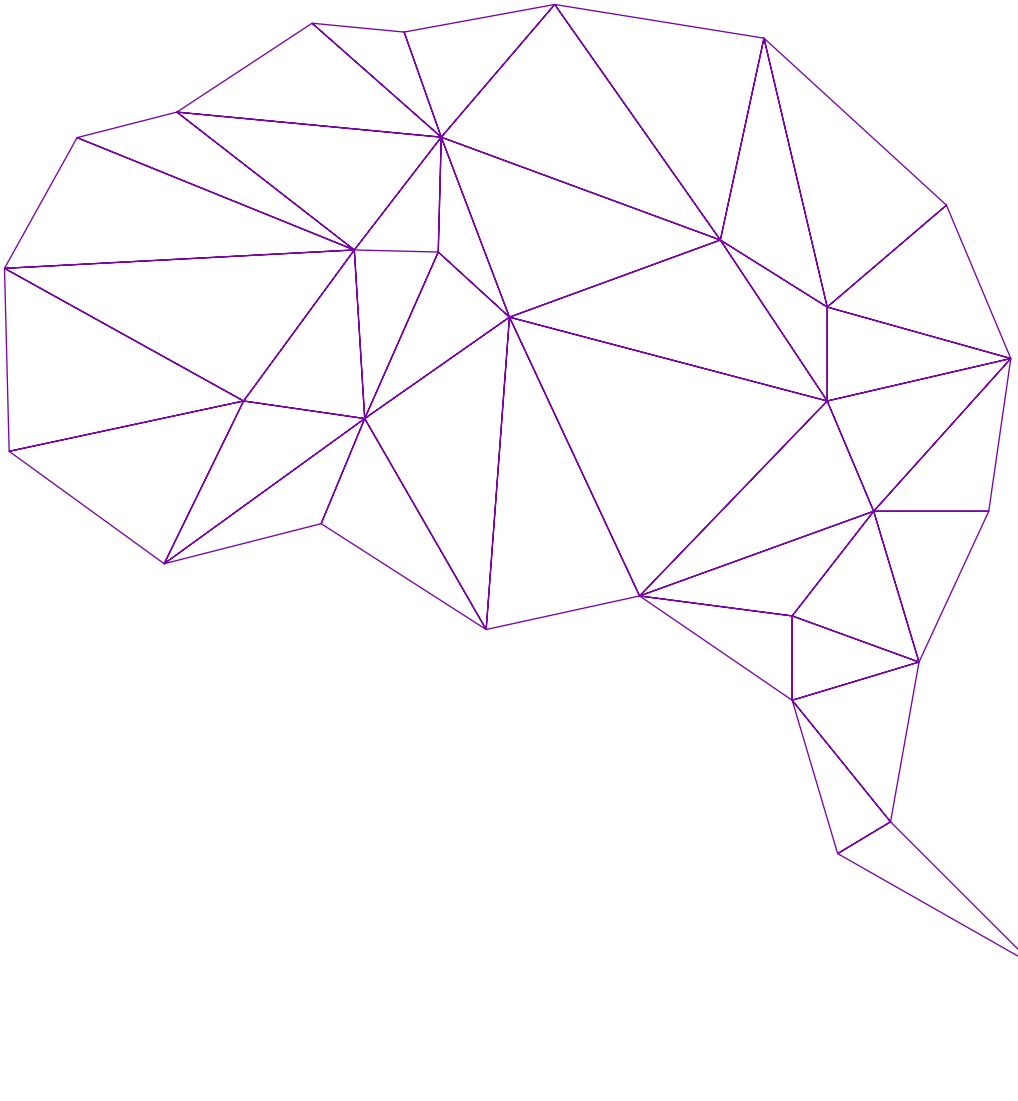

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