3. DIFFERENTIAL DIAGNOSIS AND PROGNOSTIC MARKERS OF STROKE

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Stroke, as the most common sequel of decreased cerebral blood flow due to embolic or thrombolytic occlusion of cerebral arteries, leads to an acute state in the neurologic patient. Laboratory diagnosis along with noninvasive examinations such as computed tomography, colour Doppler and nuclear magnetic resonance provides basic data for timely recognition and management of patients with acute cerebrovascular disturbance. Although the neuroimaging techniques enable almost all diagnoses of neurologic disease, laboratory findings remain a sensitive indicator of the current state of an individual allowing for identification of pathologic findings, assist in reaching the diagnosis, are useful in therapeutic success monitoring, and enable the disease outcome to predict.

Laboratory diagnosis in patients with acute neurologic disturbance includes simultaneous examination of the blood, urine and cerebrospinal fluid (CSF). Analysis of CSF provides valuable information for the differential diagnosis of stroke, primarily to distinguish between ischaemia and haemorrhage, and assists in recognizing artificial, puncture-induced haemorrhage and other neurologic diseases such as meningitis, abscess, metastases, etc., that may be masked as a cerebrovascular disease. The cytologic and biochemical analyses of CSF indicate the stage of pathologic process, size of lesion, and metabolic status of cerebral tissues.

Analysis of CSF, which should be done within the shortest possible time, includes physical examination, cytologic examination, i.e. determination of cell count and type, and haematogenous pigment examination. Additional CSF analysis includes determination of protein content, glucose and lactate concentrations, and electrolytes.

3.1   Physical examination

In physiological conditions, CFS is clear and colourless. It may be pink or red if many red blood cells are present, and cloudy in the presence of white blood cells more than 400 elements/mm3, or very high protein content. When the blood in CSF persists for more than four hours, xanthochromia may have occurred due to the presence of haemoglobin pigment from lysed red blood cells. Protein levels higher than 1.5 g/L may produce yellowish discoloration that can stimulate xanthochromia of red blood cell origin.

Irrespective of the findings obtained by physical examination of the CSF, i.e. whether it is clear, colorless, xanthochromic or haemorrhagic, spectrometric analysis of the absorption spectra is recommended.

3.2   Spectrometry of CSF

Spectrometric analysis of haematogenous pigments in the CSF is a highly sensitive method enabling detection of microhaemorrhage and intracerebral haemorrhage or subdural haematoma. Xanthochromia may be caused by one of three substances: bilirubin, oxyhaemoglobin or methaemoglobin. CSF spectrophotometry should be used in suspected intracranial vascular disorders and appears to be a good marker in the early stages of haemorrhage, when pathological changes are not yet detectable by cytology. In comparison with computed tomography imaging, it is a good, reliable and complementary method that can establish a specific diagnosis in every patient. The method also allows for monitoring of the course of the pathologic process, which can initially be ischaemic and later haemorrhagic.

Bilirubin and oxyhaemoglobin have been shown to cause xanthochromia in subarachnoid haemorrhage, i.e haemorrhagic stroke. The finding of oxyhaemoglobin usually indicates a recent haemorrhage, however, it may also be an artifact due to puncture induced vascular lesion. The finding of methaemoglobin or methaemoglobin with oxyhaemoglobin definitely points to intracerebral or subdural haemorrhage, intracerebral or subdural haematoma, or haemorrhage adjacent to skeletal musculature or from an aneurysm.

3.3   Cytology of CSF

Differentiation between ischaemic and haemorrhagic stroke can be made on the basis of cell count and type. In ischaemic stroke, CSF cytology shows a normal or mildly elevated cell count, whereas in case of haemorrhagic stroke the erythrocyte to leukocyte ratio only initially corresponds to the ratio found in peripheral blood. In the early stage, i.e. in the first 12 hours of stroke, leukocytes are on an increase due to meningeal irritation, and so is the number of polymorphonuclear granulocytes, which may rise to 500 polymorphonuclear leukocytes and lymphocytes per cubic mm in a few days due to red cell degradation. In the later stage, polymorphonuclear leukocytes show a decrease, whereas the number of mononuclear cells increases, because polymorphonuclear leukocytes have gradually disappeared and lymphocytes remain in the CSF.

In addition to the haematogenous pigment analysis, cytologic examination of the CSF contributes to the accuracy of the differential diagnosis of cerebral ischaemia. Subarachnoid haemorrhage can be ruled out, and the presence of the

inflammatory component (meningitis, brain abscess) or neoplasms (intrameningeal metastases) can be indicated by use of cell analysis.

3.4 Biochemical markers in CSF

3.4.1 Total proteins

Determination of protein concentration in the CSF alone is a sensitive marker. However, protein determination along with CSF cytology contributes to the accuracy of the differential diagnosis of stroke, especially haemorrhagic stroke. In case of subdural haemorrhage, protein concentration and erythrocyte count show an almost parallel increase whereas, in case of intracerebral haemorrhage with CSF involvement, the protein concentration shows an unproportionally mild increase relative to the very high red blood count. Normal or very rarely elevated protein and cell findings are indicative of intracerebral haemorrhage without CSF involvement.

3.4.2 Glucose and lactate

Determination of glucose and lactate concentrations in CSF provides useful data on the brain metabolic status. Ischaemia and haemorrhage reduce the cerebral glucose supply, and glucose anaerobic metabolism, i.e. glycolysis, increases, resulting in elevated lactate concentration. Therefore, a low glucose concentration and high lactate concentration point to pathologic tissue changes, the degree of pathological change being determined by the length, size and localization of the lesion. Glucose is the basic source of energy for CNS cells. It is necessary simultaneously to determine glucose level in the blood and in the CSF because the blood glucose level affects CSF glucose level. Intravenous glucose therapy in diabetic patients and hypoglycaemia can influence glucose levels in CSF.

The level of lactate in CSF is not influenced by the plasma concentration. The concentration of lactate in CSF is a reflection of anaerobic metabolism and is an indicator of the outcome following perinatal hypoxia. Newborn infants with perinatal hypoxia and poor outcome (death or permanent neurologic defect) have significantly higher lactate levels (4.5 vs. 2.5 mmol/l). However, the measurement of lactate concentration enables differentiation between a reversible and an irreversible lesion.

In patients with subarachnoid haemorrhage, trauma to the central nervous system or stroke, the concentration of lactate correlates with the disease outcome. In patients with permanent neurologic defect, the concentration of lactate remains increased for 18 to more than 48 hours, whereas a lactate concentration showing normalization within 48 hours points to a more favorable outcome. Lactate values showed good correlation with Glasgow Coma Scale.

Although diagnostic possibilities in neurologic emergency have greatly improved, neither usual laboratory diagnosis nor CT or NMR can differentiate the lesions that lead to reversible functional deficiency from those that are associated with irreversible defects, nor can completely meet the needs in predicting stroke outcome. Therefore, new biochemical tests have been constantly investigated and proposed, in order to contribute to more accurate diagnosis, therapeutic monitoring, and neurologic outcome predicting. Of recent CSF tests, mention should be made of those substantiated by results from numerous clinical studies, i.e. LDH, CKBB, NSE and S-100 protein.

3.4.3 Lactate dehydrogenase (LDH)

The measurement of LDH in CSF, known as a marker of cell death, has proved highly clinically useful in both perinatal and cerebral ischaemia. Among newborn infants with perinatal ischaemia followed-up for 15 months, the group with neurologic lesions had significantly higher LDH values measured within 24 hours from birth as compared with those without such lesions and the control group. The high LDH sensitivity in cerebral ischaemia has been explained by the long LDH half-life in CSF and higher intracellular concentration. Some studies have shown the values of LDH measured within 8 hours from stroke to be significantly higher in patients with neurologic defects than in those with transient ischemic attack that entailed no neurologic damage.

These authors have also demonstrated that LDH concentration correlates with the site and size of infarct.

3.4.4 Creatine kinase – BB isoenzyme (CKBB)

The CKBB isoenzyme is found in high concentration in cerebral tissue, where it is evenly distributed and intracellularly located. The experience acquired to date shows that it is a very sensitive marker of organic tissue damage, the dynamics of its release depending on the type of lesion (ischaemia or trauma). In ischaemia, the optimal time for CSF analysis is between 24 and 72 hours (maximal enzyme release). Thus, a significantly increased CKBB activity within 24 to 72 hours from stroke points to ischaemia, whereas significantly elevated values within several hours are indicative of trauma.

An elevated enzyme activity persisting over a prolonged period of time points to pathological process progression and the development of secondary lesions.

3.4.5 Neurone specific enolase (NSE)

NSE, gg dimer, is an isoenzyme of the glycolytic enzyme enolase, which is primarily found in neuronal cytoplasm. NSE reaches peak values on day 3-5 of the disease onset. The CSF concentration of NSE correlates with the size of stroke and indicates postischaemic damage and cell death. It is not associated with functional recovery. The primary NSE increase in serum is followed by a secondary, usually less pronounced increase, which is a consequence of secondary brain tissue lesions due to oedema and elevated intracranial pressure. This secondary NSE increase can precede the occurrence of clinical signs that point to the progressing neurologic lesion.

A correlation has been shown to exist between the lesion extent (CT) and NSE in CSF and serum. A NSE concentration exceeding 50 ng/ml points to a poor disease outcome. NSE also appears to be a sensitive marker of cerebral tissue (neuron) lesion caused by ischemia. However, elevated NSE values have also been recorded in epilepsy patients, thus additional studies of NSE sensitivity and specificity in ischemia are needed.

3.4.6 S-100 protein

S-100 protein of 21000 Da is an acidic calcium-binding protein found in brain tissue, primarily in the cytoplasm of astroglial cells, and released into the CSF upon cell death. It consists of the a- and ß- subunits. Its elevated serum concentration after ischaemic stroke points to glial cell necrosis as well as to a blood-brain barrier damage. Recent data show the concentration of S-100 protein to increase between 8 hours and 4 days of stroke, to correlate with the infarct size, and to be useful in predicting clinical

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outcome in patients with subarachnoid haemorrhage. Patients with S-100 protein levels greater than 100 µg/l had poor outcome, whereas in those with S-100 protein levels lower than 20 µg/l post-stroke survival free from complications was observed.

Continuous measurement of S-100 protein and NSE concentrations in patients with acute ischaemic stroke revealed the blood concentration of S-100 protein during acute ischaemic stroke to be a marker of infarct size and useful in outcome prediction.

Periodical measurement of S-100 protein concentration for 10 days of stroke allows for the extent of stroke to assess and long-term neurologic outcome to predict with greater accuracy than by periodical measurement of NSE concentration in blood.

However, it seems that continuous measurement of S-100 protein concentration in blood might be useful in monitoring therapeutic effects in cerebrovascular diseases.

3.4.7 Myelinbasic protein (MBP)

MBP is a brain specific protein and one of the components of myelin, a lipid substance that forms a multi-layered axon sheath. Myelin is formed by oligodendrocytes and consists of their cell membrane that wrap themselves around axons. MBP is more specific for CNS destruction than for inflammatory processes, but is not specific for the aetiology of CNS damage. Like S-100 protein and NSE, this protein also reaches its peak values on day 4-5 of stroke, which correlate with lesion size and short-term clinical outcome. In patients with peak MBP <5 µg/L no neurologic deficits were identified, whereas those with MBP >10 µg/L died or were disabled.

3.4.8 Thrombomodulin (Tm)

Thrombomodulin is one of the important vasoprotective molecules. It is a transmembrane protein expressed primarily in endothelial cells. Thrombomodulin inhibits procoagulant activity of thrombin and redirects its substrate specificity toward the activation of protein C and fibrinolysis inhibitor.

A protocol for the diagnosis and characterization of stroke, which includes NSE, S-100 protein, MBP and thrombomodulin markers, has been developed and patented by Jackowski in 2000 (Stroke Panel, SYNX Pharma Inc., Canada). A combination of markers provides more complete and reliable data than a single marker determination. At least one of the markers is elevated on admission. Peak levels of NSE, S-100 protein, MBP but not thrombomodulin significantly correlate with the admission National Institute of Health Scale Score (NIHSS). Similarly, peak levels of NSE, S-100 protein, thrombomodulin but not MBP significantly correlate with discharge modified Rankin scale scores.

In 1996, FDA approved the use of thrombolytic therapy by recombinant thromboplastin for certain stroke subtypes, which has been experimentally performed since 1992. The matrix metalloproteinase-9, a novel biochemical marker of the risk associated with recombinant thromboplastin therapy, helps in deciding on the use of the thrombolytic.

3.4.9 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases play a key role in remodeling extracellular matrix. It is a family of extracellular soluble or membrane-bound proteases. Serine protease can convert proactive MMPs to active forms. In physiologic conditions, the activity is also controlled by tissue inhibitors of MMPs. Recent reports show the increase in plasma MMP-9 levels to be an independent risk factor

for haemorrhagic transformation in all stroke subtypes. However, plasma levels of MMP-9 were significantly higher only in patients who developed haemorrhagic transformation, whereas in patients without haemorrhage the increase was not significant. These data suggest that MMP-9 does not play an important role in early pathophysiologic events in cerebral ischaemia, however, it may play a role in platelet mediated thrombus formation which can result in ineffective thrombolysis. High plasma MMP-9 levels might be associated with thrombolytic resistance.

Increased MMP-9 levels are a potential plasma biomarker of thrombolysis failure in stroke and a strong predictor of haemorrhagic transformation and worse outcome.

The fact is that cerebrovascular diseases are the most common disorders of the central nervous system, and the third cause of death in industrialized countries, immediately following cardiac and malignant diseases. Cerebral vasculature and cerebral blood flow play a key role in the onset of cerebrovascular diseases. However, the complex course of the disease onset and development has not yet been fully clarified. The modifiable risk factors, diseases, and states that are risk factors for stroke are presented in Table 1. Therefore, the laboratory diagnosis of neurological patients has been ever more focussed on the detection of risk factors in order to prevent cerebrovascular disease in general and stroke in particular by their reduction and control.

Table 1. Risk factors for stroke

Modifiable	Non-modifiable	Diseases and states - risk factors for stroke
Cigarette smoking	Age	Arterial hypertension
alcohol and other dependence substance abuse	Sex	Cardiac diseases
overweight and physical inactivity	Race	increased serum cholesterol
stress	stroke or TIA in family or personal history	diabetes mellitus
oral contraceptives		

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