appendix e-1

Analysis of soluble interleukin-2 receptor as cerebrospinal fluid biomarker for neurosarcoidosis

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Details of patients included in this work

23 patients with a diagnosis of definite or probable neurosarcoidosis according to the Zajicek-criteria were included in the study.[1] Diagnosis of sarcoidosis was histologically proven in 21 patients, 17 of which had a systemic biopsy and 4 a CNS biopsy.

Clinical disease activity of patients with neurosarcoidosis at the time of CSF withdrawal was assessed by experienced neurologists and categorized as either "clinically active disease" or "clinical remission." Clinically active disease was defined as new clinical symptoms or signs or worsening of pre-existing symptoms or signs as compared to a previous neurological assessment. Remission was defined as absence of clinical symptoms or signs or clinical disease stability or improvement as compared to a previous neurological assessment. According to these definitions, data from 15 CSF/serum samples from 12 patients with active disease and data from 27 CSF/serum samples from 18 patients with neurosarcoidosis in clinical remission were available for analysis. 13 of the 15 CSF/serum samples from patients with clinically active disease were collected while patients received immunotherapy including corticosteroids (n = 5), corticosteroids and cyclophosphamide (n = 1), azathioprine (n = 1), corticosteroids and methotrexate (n = 5) and corticosteroids and plasma exchange (n = 1). 19 of the 27 CSF/serum samples from patients in clinical

remission were collected while patients were under immunotherapy including azathioprine (n = 1), corticosteroids and azathioprine (n = 6), corticosteroids (n = 6), corticosteroids and methotrexate (n = 4), corticosteroids, methotrexate and infliximab (n = 1) and corticosteroids, azathioprine and cyclophosphamide (n = 1).

Furthermore, based on cranial magnetic resonance imaging (MRI) findings at the time of CSF withdrawal, patients with neurosarcoidosis were grouped into those with (n = 11) and those without (n = 4) diffuse leptomeningeal gadolinium enhancement on cranial MRI, as described previously.[2]

115 patients with non-inflammatory neurological diseases (NINDs) served as controls. Patients with NINDs had to have a normal cerebrospinal fluid (CSF) cell count (≤ 4/µl) and no CSF-specific oligoclonal bands, but were chosen to have a wide range from normal to severely elevated CSF/serum albumin quotients. Diagnoses of patients with NINDs were headache (n = 21), cranial nerve disorders (n = 7), dementia (n = 6), encephalopathy (n = 4), functional disorders, psychosomatic or psychiatric disorders (n = 17), movement disorders (n = 7), polyneuropathy (n = 12), seizures (n = 12), sleep disorder (n = 3), spinal stenosis (n = 11), cerebrovascular disease (n = 10), motor neuron disease (n = 2), ataxia (n = 1), transient global amnesia (n = 1) and adenocarcinoma of lung with brain metastases and ventriculoperitoneal shunt (n = 1). All diagnoses were established by experienced neurologists.

We also included 18 patients with viral meningitis and 9 patients with acute bacterial meningitis. In patients with viral meningitis, diagnoses were based on typical clinical and CSF findings and the PCR detection of varizella zoster virus DNA (n = 9) or

enterovirus RNA (n = 9) in CSF. In patients with bacterial meningitis, *streptococcus pneumoniae* (n = 3) or *neisseria meningitidis* (n = 2) was detected in CSF. While the remaining 4 patients had typical clinical and CSF findings of bacterial meningitis, a causative agent could not be identified in these patients.

In patients with neurotuberculosis (n = 8), diagnosis was based on clinical findings, characteristic MRI and CSF findings as well as PCR detection of *mycobacterium tuberculosis* in CSF in 4/8 patients. Clinical manifestations of neurotuberculosis were meningoencephalitis (n = 5), meningitis (n = 1), meningitis with abducens nerve palsy (n = 1) and meningomyelitis with conus/cauda syndrome (n = 1).

Diagnosis of Guillain-Barré syndrome (n = 8) was based on typical clinical and electrophysiological as well as CSF findings.

Among patients with multiple sclerosis (MS, n = 19), 17 had a diagnosis of relapsing-remitting MS according to the McDonald 2017 criteria.[3] 1 patient had secondary progressive MS and 1 patient had a clinically isolated syndrome. All of these 19 patients had an intrathecal IgG synthesis as evidenced by the presence of CSF-specific oligoclonal bands or by the detection of an intrathecal IgG synthesis in CSF/serum quotient diagrams. We considered patients with MS to have active disease at the time of lumbar puncture if they had a clinical relapse and/or at least one gadolinium enhancing lesion on MRI within a time interval of 30 days before lumbar puncture. According to this definition, 13 of 19 patients with MS included in our study had active disease at the time of lumbar puncture.

We also measured soluble interleukin-2 receptor in CSF and serum samples of 13 patients with primary CNS lymphoma and 2 patients with systemic lymphoma with CNS involvement. 2 of those 15 patients were HIV positive. In these 2 patients, HIV viral load in blood and CD4 helper cell count were 230.000 copies/ml and 61 cells/µl, and 131.000 copies/ml and 47 cells/µl, respectively. Diagnosis of CNS lymphoma was based on clinical and MRI findings and histopathological examination of a brain biopsy in all 15 patients.

References

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