**Pleocytosis is not fully responsible for low cerebrospinal fluid glucose in meningitis**

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**Supplemental methods**

We used the University of California, San Francisco (UCSF) central clinical laboratory database to identify CSF samples with inflammation in patients one year of age or older collected between 2009 and 2015. We also identified samples from the UCSF neuro-infectious disease and UCSF neuroimmunology sub-specialty clinics. We did not include CSF samples from infants less than one year old as their blood-brain barrier is not mature and could unduly confound our results. Inflammatory CSF was defined as a pleocytosis (≥ 6 x106 cell/L) with or without abnormal absolute glucose level. Only the initial CSF sample for each patient at presentation was included in the main analysis. Repeat samples belonging to the same patient were also included for longitudinal analyses if the samples had been drawn at least 7 days apart.

In total, we screened 1236 CSF profiles and reviewed 492 charts (see flowchart). 244 charts were excluded on the following grounds: CSF obtained intra-operatively, in the context of extra-ventricular drain placement or routine sampling in neuro-ICU patients; in the context of treatment of hydrocephalus, pseudotumor cerebri or intrathecal medication administration; CSF obtained for tumor evaluation or neoplastic meningitis, surgical wound infections, or when alternative source of sepsis were identified; cases with delayed presentation to our hospital (e.g. follow-up in specialty clinic after >1 month of disease, for chronic meningitis or already on antibiotics for ≥ 24 hours at the time of the LP for acute meningitis); samples with concomitant systemic hypoglycemia (serum glucose < 40 mg/dL). We did not include oncologic cases as the mechanisms of cancerous meningitis were beyond the scope of this study. We did not include multiple sclerosis cases, as they are numerous, often present low degrees of inflammation and may have skewed the linear relationship with CSF glucose. Samples with isolated abnormalities without pleocytosis such as low glucose level (< 40 mg/dL), high protein (≥ 50 mg/dL), red cells (≥ 6 x106 cell/L), IgG index (≥0.7) or oligoclonal bands (≥3) were excluded. To avoid confusion, we did not consider missing symptoms on the classical meningitis triad (fever, neck stiffness and headaches) as an exclusion criteria as this triad is often absent, even in severe bacterial meningitis [1]. Therefore, our definition of meningitis is laboratory based (i.e. pleocytosis is present). After exclusion, 248 subjects remained for chart review, of which 23 were discarded due to uncertain etiological diagnoses even after in-depth review. 225 remaining cases met our inclusion criteria and had a definite etiological diagnosis defined as follows:

* Proven infectious diagnosis by identification of an infectious agent by culture, PCR, antigen or antibody testing; identification of specific pathology on brain biopsy (e.g. granulomatous inflammation in sarcoidosis and absence of infectious agent);
* A minority of the cases (~18% of the cohort) was not proven by a test, but clinical certainty about the etiology was established, documented in the notes and subsequent therapeutic action leading to improvement and no change in the diagnosis over at least one follow-up visit. This represented 13.6% of probable bacterial meningitis (e.g. post-surgical infection that responded to antibiotics, but culture data was lacking) and 22.6% of aseptic meningitis (e.g. probable viral meningitis with clear documentation of a sick contact, a viral prodrome and response to conservative treatment, but definitive testing not sent);
* For autoimmune etiologies, clinical experts in the field documented the diagnosis in their notes, based on imaging and clinical features.

We dichotomized the diagnoses into septic (bacterial, n=68, fungal, n=29, atypical bacteria, n=8, mycobacterial, n=3, parasite, n=2 cases of toxoplasmosis) and aseptic meningitis (immunological, n=63, viral, n=49, chemical-pharmacological, n=3). See table 2 for details. Of the 225 unique cases in our sample, 64 had serial repeat CSF sampling within a month and were used for longitudinal analyses.



**Supplemental Results**

We show the relationship between serum and CSF glucose across our entire dataset according to the delay between CSF and serum collection (supplementary figure 1). One can see that the variance is high in this cohort with varying degrees of CSF inflammation and presence or absence of a pathogen, but that it does not increase with increasing delays.



**Supplementary Figure 1:** relationship between serum and CSF glucose across our entire dataset stratified by delay between collection of CSF and serum glucose in hours (hrs).

We performed univariate linear regressions on CSF glucose and CSF glucose ratio to identify variables to include in the multi-variate model (Table 1).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | CSF Glucose  (n=225) | | | CSF-to-serum glucose ratio (n=172) | | |
|  | b | p-value | adj-R2 | b | p-value | adj-R2 |
| Age |  | NS |  |  | NS |  |
| Sex |  | NS |  | -0.06 | <0.05 | 0.023 |
| HIV |  | NS |  |  | NS |  |
| Diabetes Mellitus | 22.2 | <0.001 | 0.071 | -0.1 | <0.05 | 0.031 |
| Remote Neurosurgery |  | NS |  |  | NS |  |
| Recent Neurosurgery |  | NS |  | -0.1 | <0.05 | 0.023 |
| Diagnosis | -21.1 | <0.001 | 0.16 | -0.19 | <0.001 | 0.25 |
| WBC |  | NS |  |  | NS |  |
| Leukocytes (log) | -11.3 | <0.001 | 0.12 | -0.1 | <0.001 | 0.2 |
| Neutrophils | -16.4 | <0.01 | 0.045 | -0.21 | <0.001 | 0.16 |
| Lymphocytes | 13.8 | <0.01 | 0.03 | 0.17 | <0.001 | 0.1 |
| Monocytes |  | NS |  |  | NS |  |
| Proteins (log) | -21.7 | <0.001 | 0.12 | -0.24 | <0.001 | 0.27 |

**Supplementary Table 1:** Univariate linear regression analysis on absolute CSF glucose and CSF-to-serum glucose ratio. b: linear coefficient, HIV: human-immunodeficiency virus, WBC: white blood cells.

We performed additional sensitivity analyses:

We fitted the same model on the dataset excluding 22 microbial cases without a documented pathogen on culture. The results were similar: we found an interaction between the septic category and the protein level with β3 = -0.28 ± 0.04, p<0.001 and β3 = -19.4 ± 5.1, p<0.001 based on regressions for the glucose ratio (adjusted-R2=0.44) and absolute glucose (adjusted-R2=0.42), respectively.

To rule out an impact of neurosurgical cases on our results, we excluded all cases with a remote or recent history of neurosurgery. The results were similar: we found an interaction between the septic category and the protein level β3 = -0.15 ± 0.07, p=0.03 and -18.0 ± 9.0, p=0.048 based on regressions for the glucose ratio (adjusted-R2=0.39) and absolute glucose (adjusted-R2=0.20), respectively.

Similarly, we checked whether the relation between CSF proteins and glucose was also present in the surgical subgroup with bacterial infection. We found a strong inverse correlation between the level of CSF proteins and glucose among the 22 cases with β = -0.29 ± 0.11, p=0.01 and β= -38.8 ± 8.4, p<0.001 based on regressions for the glucose ratio (adjusted-R2=0.27) and absolute glucose (adjusted-R2=0.53), respectively.

Additionally, we investigated the relationship between CSF proteins and glucose in the two types of aseptic meningitides separately: inflammatory (e.g. autoimmune) and infectious (i.e. viral, see table 2). We found no correlation between CSF proteins and glucose or glucose ratio in the inflammatory, or in the infectious cases of aseptic meningitis (p > 0.1 for all).

Because one may argue that neutrophilic pleocytosis in septic cases may be an important driver, we compared similar degrees of inflammation as defined by the absolute neutrophil count and found similar effects on glucose metabolism as reported above; there was no significant difference in glucose (43±36 vs 65±20 mg/dL, p=0.26, t-test) with low levels of neutrophils (n=5 and 5, 32±6 vs 35±11 mio/L, p=0.59, t-test), but decreased glucose in septic relative to aseptic cases (42±19 vs 57±16 mg/dL, p=0.046, t-test) with higher levels of neutrophils (n=9 and 23, 142±100 vs 160±124 mio/L, p=0.69, t-test).

**Supplementary table 2:** Stratified analysis for three degrees of inflammation as defined by the CSF protein levels using absolute glucose or the CSF:serum glucose ratio as the dependent variable. Proteins and absolute glucose are in mg/dL

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Low CSF Protein**  **(50-100 mg/dL)** | | | **Medium CSF Protein**  **(101-180 mg/dL)** | | | **High CSF Protein**  **(181-500 mg/dL)** | | |
| Absolute Glucose |  | n | Proteins | Glucose | n | Proteins | Glucose | n | Proteins | Glucose |
| Aseptic | 39 | 71±13 | 65±22 | 28 | 133±26 | 55±15 | 10 | 281±87 | 57±27 |
| Septic | 23 | 75±13 | 52±31 | 26 | 142±24 | 39±26 | 29 | 275±82 | 33±23 |
| T-test |  | p=0.3 | p=0.07 |  | p=0.2 | **p=0.01** |  | p=0.8 | **p=0.008** |
| Glucose ratio |  | n | Proteins | G ratio | n | Proteins | G ratio | n | Proteins | G ratio |
| Aseptic | 30 | 70±12 | 0.58±0.13 | 20 | 133±25 | 0.50±0.14 | 5 | 267±73 | 0.53±0.17 |
| Septic | 14 | 73±13 | 0.44±0.15 | 18 | 145±23 | 0.38±0.10 | 20 | 263±77 | 0.28±0.13 |
| T-test |  | p=0.4 | **p=0.003** |  | p=0.2 | **p=0.007** |  | p=0.9 | **p=0.001** |

References

1 Gaieski DF, Nathan BR, O'Brien NF. Emergency Neurologic Life Support: Meningitis and Encephalitis. *Neurocrit Care* 2015;**23 Suppl 2**:S110–8. doi:10.1007/s12028-015-0165-2