Author’s response to reviews

Title: Neutrophil CD64 index in cerebrospinal fluid as a marker of bacterial ventriculitis in children with external ventricular drainage

Authors:

Mojca Groselj-Grenc (mojcagg@gmail.com)

Andreja Natasa Kopitar (andreja-natasa.kopitar@mf.uni-lj.si)

Metka Derganc (metka.erganc@mf.uni-lj.si)

Maja Pavcnik (maja.pavcnik@kclj.si)

Version: 4 Date: 07 Apr 2019

Author’s response to reviews:

Response letter for manuscript: Neutrophil CD64 index in cerebrospinal fluid as a marker of bacterial ventriculitis in children with external ventricular drainage (BPED-D-17-00653R3)

Textual overlap:

Method section, particularly Sample collection and measurements and Statistical analysis were rewritten:

1. We added reference [26] after the sentence: Expressions of CD64 and CD163 on neutrophils, monocytes and lymphocytes were measured by quantitative flow cytometry with a FACSCanto flow cytometer (Becton Dickinson, CD, USA) using the Leuko64TM assay (Trillium Diagnostics, LLC, Brewer, Maine, USA)

2. The sentences: The assay is composed of three antibodies with specificities to CD64 (clones 22 and 32.2, both fluorescein isothiocyanate conjugated) and to CD163 (clone Mac2-148, phycoeritrin conjugated), and a fluorescence bead suspension with three fluorescence signals (green fluorescence due to fluorescein isothiocyanate, orange fluorescence similar to phycoeritrin, and red fluorescence of starfire red) for unique identification of beads, and used for instrument calibration and standardization of leukocyte CD64 and CD163 expression. Mean fluorescence intensity was measured as a linearized value of log scale on lymphocytes (red, negative control, measuring CD64 expression), monocytes (green, positive control, measuring CD64, and CD163 expression), neutrophils (blue, measuring CD64 expression), and beads (aqua
blue, measuring fluorescein isothiocyanate, and phycoeritrin expression). were deleted, and in details was added in the next sentence with reference: The sample preparation (50 µl of CSF) and flow cytometer setup were based on the manufacturer’s instructions and are described in details in our previous paper [26].

3. The next two sentences were changed (underlined):

Index calculations were performed using Leuko64 QuantiCalc software (Trillium Diagnostics, LLC, Brewer, Maine, USA) and it was measured as a ratio of linearized mean fluorescence intensity of the cell population to the fluorescein isothiocyanate signal from the beads. Automated measurement of the lymphocyte CD64 index, which had to be less than 1.0, served as an internal negative control of the assay, and automated measurement of the monocyte CD64 index, which had to be more than 3.0, served as an internal positive control [26].

4. Statistical analysis

We started with the sentence: Statistical analysis was performed using MedCalc for Windows, version 17.0.4 (MedCalc Software, Ostend, Belgium). Data were presented as the median with interquartile range (IQR).

Other changes are underlined:

T-test for normally distributed data and Mann-Whitney test for not normally distributed data were used for comparing parametric variables between groups. Chi-square test was used for comparing nonparametric variables between groups. Receiver operating characteristic (ROC) curves for each studied marker were constructed to determine the optimal sensitivity, specificity, positive and negative predictive value, cut-off value and diagnostic accuracy from the area under the ROC curve (AUC).

Ethics approval and consent to participate

As we already stated ethics committee approved this procedure without a need for any consent, since CSF in patients with EVD is material to be discharged. As children in our hospital are hospitalised with parents, we explained all procedures with them, as we did with our study.

E-mail address is now matching.

P-value

0.00 was reported as <0.01
Best regards,

Mojca Groselj-Grenc