

MONITORING OF NATALIZUMAB THERAPY WITH CEREBROSPINAL FLUID ACTIVATION MARKERS

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BACKGROUND

Natalizumab is a FDA-approved medication for the treatment of active multiple sclerosis (MS). Currently, response to the medication is assessed by clinical assessment and brain magnetic resonance imaging (MRI). Cerebrospinal fluid (CSF) activation markers may be additional parameters that could help determine medication efficacy and may provide further insights into the physiological effects of natalizumab.

OBJECTIVE

To determine if natalizumab treatment is effective in reducing CSF disease activity markers.

METHODS

Sample Collection

CSF samples were obtained from MS patients at the International Multiple Sclerosis Management Practice (IMSMP) undergoing treatment for Natalizumab through an IRB-approved protocol. CSF samples were collected at baseline, and at 6 and 12 months post-treatment, by lumbar puncture or access port aspiration of the Medtronic pumps. All samples were coded and aliquots were frozen at -80°C until analysis. Informed consent was obtained from all patients.

Biomarker ELISA analysis

Levels of Fetuin-A, osteopontin, and nitric oxide samples in the CSF were determined by ELISA at baseline, 6 months and 12 months post-treatment following the manufacturer's instructions. The analytical limit of detection is <1ng/mL, <0.312ng/mL and <1.56umol/L for Fetuin-A, osteopontin and nitric oxide respectively. ELISA plates were measured via absorbance at 450nm for Fetuin-A and osteopontin and 540 nm for nitric oxide. Concentrations of diluted samples were then read off the standard curve constructed by plotting the absorbance values against each respective standard level using a four-parameter function for both Fetuin-A and osteopontin and a linear function for nitric oxide. Statistical analysis was performed using paired t-test.

RESULTS

The characteristics of the 79 MS patients who received natalizumab treatment is shown in Table 1. The percentages and mean levels of Fetuin-A, osteopontin, and nitric oxide as well as average total cell count and number of cells/mL at baseline, 6-months post-treatment, and 12-months post-treatment are shown in Table 2 and Figure 1 respectively.

Table 1. Patient Demographics

Determinants	
Number	79
F/M (ratio)	51/28 (1.8)
Mean Age	46 years
Age Range	23-75 years
Disease Duration Range	3-34 years

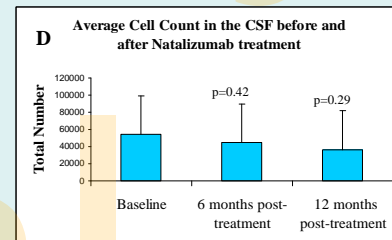
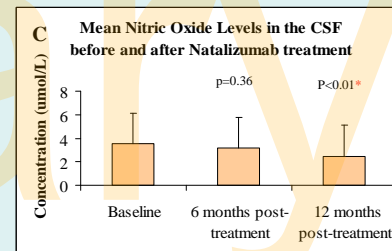
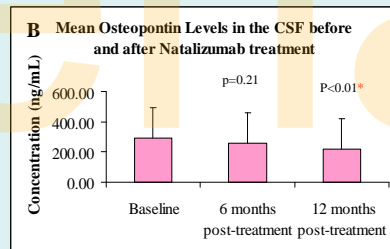
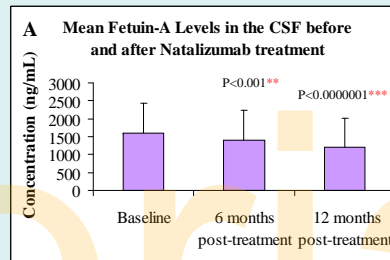
Disease Activity Marker Analysis

Natalizumab reduced total cells counts, levels of CSF osteopontin, nitric oxide and fetuin-A in approximately two-thirds of all patients after one year of treatment (Table 2). There was, however, no statistical significance in the pre-and post-treatment values for cell count, osteopontin and nitric oxide (Figure 1) at 6 months. At one year, pre-and post-treatment values for Fetuin-A, osteopontin and nitric oxide were significantly reduced (Figure 1), but there was no statistical significance in cell count. Fetuin-A had a mean of pre-treatment level of 1601.3 ng/mL and this was reduced at six months at 1409.3 ng/mL ($p < 0.01$) and at a year to 1195.4 ng/mL ($p < 0.0000001$) (Figure 1A). In addition, CSF Fetuin-A levels in individual patients correlated with disease activity as determined by clinical relapses and brain MRI activity. Overall at six months 65% of treated patients had reduced levels and by one year 77% were affected (Table 2). Meanwhile, osteopontin had a pre-treatment value of 290.6 ng/mL and this was reduced at one year to 217.6 ng/mL ($p < 0.01$) (Figure 1B). Nitric oxide had a pre-treatment value of 3.6 umol/L and this was reduced at one year to 2.5 umol/L ($p < 0.01$) (Figure 1C).

Table 2. Effect of Natalizumab in CSF activity markers and cell count in the CSF of MS patients receiving natalizumab treatment over 12 months

	% decreased at 6 months post-treatment	% decreased at 12 months post-treatment
Fetuin-A (ng/mL)	65%	77%
Osteopontin (ng/mL)	54%	63%
Nitric Oxide (umol/L)	54%	67%
Total Cell Count	59%	71%
Cells/mL	53%	61%

Figure 1. Mean Levels of CSF disease activity biomarkers decrease among MS patients receiving Natalizumab treatment over 12 months.



CONCLUSIONS

Our data suggests natalizumab treatment reduces disease activity biomarkers and total cell counts in the CSF.

Of the biomarkers investigated, CSF Fetuin-A appears to be most significantly reduced by natalizumab treatment. This may have utility in effective, therapeutic decision-making.

The reduction of CSF levels for all the biomarkers is more marked after 12 months of treatment when compared to 6 month values.

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