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Review

Urine: An under-studied source of biomarkers in multiple sclerosis?

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ABSTRACT

There remains a need for sensitive and reliable biomarkers that can be used longitudinally in multiple sclerosis. Whilst both CSF and MRI have been extensively studied, they remain invasive and expensive methods of investigation. On the contrary, urine provides a valuable fluid which is readily available for serial sampling. Some work has been done on urinary biomarkers in multiple sclerosis; however, urinary biomarkers have not been extensively studied and validated for use in routine clinical practice, and urine remains understudied and underutilised. In this review the use of neopterin, urinary free light chains, nitric oxide metabolites and urinary myelin basic protein-like protein as potential biomarkers that have been identified in urine are discussed, and avenues for future study are raised.

Contents

1. Introduction

Urine provides a valuable fluid readily available for serial sampling in multiple sclerosis (MS), but it is understudied and underutilised (Smith and Lassmann, 2002). In contrast to CSF, urine has the distinct advantage of being easy to collect, and frequent and repeated sampling is easy to achieve. In addition, substances which are excreted in the urine are often present in higher concentrations than in CSF or blood due to fractional excretion; a result of both glomerular filtration and water resorbtion in the renal tubule (Giovannoni and Thompson, 1998). A single urine sample represents urinary excretion over a number of hours due to urine storage in the bladder (Giovannoni and Thompson, 1998). Whilst CSF oligoclonal bands are frequently used in the diagnostic work-up for MS, they are rarely, if ever, used for disease and/or treatment monitoring, not least because to date a clinical response has not been correlated with a qualitative change in the number or pattern of oligoclonal bands (Awad et al., 2010). Additionally, obtaining serial CSF samples requires repeated lumbar puncture, which many patients may not find acceptable (Bielekova and Martin, 2004). The absence of a sensitive and specific serum antibody in MS (Graber and Dhib-Jalbut, 2011) has limited the use of serological biomarkers, although the search continues.

A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (Bielekova and Martin, 2004). Although biomarkers do not by definition have a causal relationship to the disease in question, they often reflect underlying pathogenesis to

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some degree. In a complex disease such as multiple sclerosis, a single biomarker may well represent only a single facet of disease pathogenesis (Bielekova and Martin, 2004). The best validated and most widely used biomarkers in MS are MRI measures of disease activity, which are commonly used in clinical trials and treatment monitoring in the outpatient clinic.

Some work has been done on urinary biomarkers in multiple sclerosis; however, urinary biomarkers have not been extensively studied and validated for use in routine clinical practice. The reasons for this lack of study are not clear, although concerns regarding frequent urinary tract infections in people with MS limiting the use of urinary markers may be one explanation. However, with the ready availability of bedside tests for urinary tract infection (as indicated by the presence of nitrites), samples can be easily evaluated for infection prior to further testing. Certainly, there are no longitudinal studies evaluating the use of urinary markers as surrogate markers against other disease outcome measures in MS. There remains a need for sensitive and reliable surrogate markers for the monitoring of disease activity in MS, and it may well be that the time has come to revisit urine as a potential source of these. In this review, potential biomarkers that have been identified in urine are discussed, and ideas for future study are raised.

2. Neopterin

Neopterin is a product of interferon-γ activated macrophages (Huber et al., 1984), and its formation is augmented by the presence of TNF-α (Werner-Felmayer et al., 1990). It has been extensively studied in the context of systemic infections, HIV, malignancies and autoimmunity (Fuchs et al., 1992). In the context of MS, it has been found in the CSF (Fredrikson et al., 1987; Ott et al., 1993; Shaw et al., 1995) and serum (Ott et al., 1993) of people with MS. Neopterin is a sensitive marker of immune activation, and serum concentrations have been used to monitor the biological effect of interferon-β (Williams and Witt, 1998; Bagnato et al., 2002), with serum levels of neopterin peaking approximately 2 day post-dose (Bagnato et al., 2002; Cook et al., 2001). No studies have examined a possible correlation between neopterin in the CSF and in the urine, although such a correlation has not been found in other disorders, although urine concentration was not controlled for (Nomoto et al., 1991).

Neopterin is a stable compound in vivo, which is excreted in the urine. When measured in the urine it should be expressed as a ratio to creatinine (or total protein) in order to control for variable urine concentrations (Giovannoni et al., 1997). It can be measured using high pressure liquid chromatography (HPLC), a standard technique (Niederweiser and Wetzel, 1982; Giovannoni et al., 1997) or ELISA. Neopterin is stable in the urine, with reproducible results produced when urine is stored at room temperature for 48 h, $\geq$72 h at 4 °C and $\geq$4 months at −20 °C (Giovannoni et al., 1997). In addition, levels have been shown to remain stable through repeated free-thaw cycles (Giovannoni et al., 1997).

Urinary levels of neopterin (expressed as neopterin:creatinine ratio to correct for variable urinary concentration) are higher in people with MS than healthy controls (Giovannoni et al., 1997); Khorami et al., 2003), with an average level of 134 (95% CI: 97–152) μmol/l in healthy controls compared to 187 (165–277) μmol/l in people with relapsing remitting MS, 218 (164–517) μmol/l in secondary progressive MS and 187 (135–231) μmol/l in primary progressive MS (Giovannoni et al., 1997). Whilst sensitivity and specificity have not been precisely calculated, urinary neopterin appears to have a relatively high sensitivity, but this is offset by a lack of specificity; levels rise in the context of a systemic inflammatory response, such as viral infection (Giovannoni et al., 1997). In addition to this, levels show increased day-to-day variability in people with MS, which is thought to reflect fluctuations in inflammatory activity (Giovannoni et al., 1997).

As noted above, as neopterin levels fluctuate in individuals in response to stimuli such as infections and inflammation, and there is utility in repeated measurements in the context of MS: in a longitudinal study, 29/31 (94%) MS patients demonstrated increased neopterin excretion compared to healthy controls during the study (Giovannoni et al., 1997) as opposed to 39/106 (37%) in a single time point cross-sectional study (Giovannoni et al., 1999). In addition there was a possible trend for people with secondary progressive MS to have higher mean urine neopterin and also greater intra-patient variability in neopterin levels than those with relapsing remitting MS (Giovannoni et al., 1997). These findings did not reach statistical significance, and it must be noted that there were only a small number of patients in this study (10 with relapsing remitting MS and 11 with secondary progressive MS).

There appears to be an increase in urinary neopterin prior to clinical relapse in people with MS, and this appears to be of greater magnitude than the day-to-day variability (Giovannoni et al., 1997). However the magnitude of these increases with relapse did not reach statistical significance, possibly due to the small number of patients that had a clinical relapse in the single study examining this (Giovannoni et al., 1997). There was no correlation between the clinical severity of relapses and the level of neopterin excretion. Urine neopterin levels appear to fall with intravenous steroid treatment (Giovannoni et al., 1997); however, only two patients received this treatment, and so caution must be exercised in interpreting this finding. Although serum neopterin levels rise in response to infection (Fuchs et al., 1992), the response of urinary neopterin to infection in people with MS was found to be variable, so at present no firm conclusions can be drawn about this, and it would need further study prior to clinical use (Giovannoni et al., 1997).

Urinary neopterin levels have also been determined on participants in a trial of interferon-β in primary progressive MS (Rejdak et al., 2010). There was a significant difference in urinary neopterin levels between those on interferon-β and placebo with a trend to increasing levels with increasing treatment duration (Rejdak et al., 2010). The reason for this paradoxical increase is unclear, but it may reflect the systemic immunological actions of interferon-β (Rejdak et al., 2010).

Neopterin therefore shows promise as a potential urinary biomarker in MS, although further work is needed in order to further examine longitudinal changes both with and without treatment. The fluctuations that are seen in neopterin levels may hinder its use, although it may well be possible to overcome these through repeated sampling. In addition, correlation with existing clinical and MRI outcome measures is required, together with further studies examining how relapses affect urinary neopterin levels.

3. Urinary FLC

People with MS have increased levels of immunoglobulin free light chains (FLC) in their CSF compared to healthy controls (Rudick et al., 1985). These excess CSF FLC, which are produced by B-cells and plasma cells within the central nervous system, can be detected using ELISA (Dobson et al., 2010a). CSF κ FLC have been shown to correlate with disability progression (Rinker et al., 2006). However, FLC cannot be detected in the serum of people with MS (Mehta et al., 1991; Tsai et al., 1992), either as a result of dilution or rapid fractional excretion by the kidneys.

It has been known for many years that urinary free light chains are present at increased concentrations in the urine of people with