Concise report

Increased monohexosylceramide levels in the serum of established rheumatoid arthritis patients

Gabriel Miltenberger-Miltenyi, Ana Rita Cruz-Machado, Jennifer Saville, Vasco A. Conceição, Ângelo Calado, Inês Lopes, Maria Fuller and João Eurico Fonseca

Abstract

Objectives. To identify serum sphingolipids that could act as candidate biomarkers in RA.

Methods. We performed lipidomic analyses in the serum of 82 participants: 19 established RA patients, 18 untreated early RA patients, 13 untreated early arthritis patients not fulfilling the classification criteria for RA, 12 established SpA patients and 20 controls. We compared the lipid levels from the different patient groups with the control group through multiple-regression analyses controlling for age at diagnosis, gender and medication (cDMARDs and corticoids).

Results. Established RA patients had significantly increased levels of sphingosine, monohexosylceramide and ceramide compared with controls, when controlling for age and gender. Monohexosylceramide levels remained significantly increased when additionally controlling for medication. On the contrary, SpA patients had significantly decreased levels of ceramide, in both analyses.

Conclusion. We observed a detectable increase in the levels of certain sphingolipids in the serum of established RA patients when compared with controls, in line with previous observations in the synovial fluid. Such findings provide further evidence that sphingolipids may play a key role in the pathophysiology of RA.

Key words: rheumatoid arthritis, serum sphingolipid levels, biomarker

Introduction

The landscape of possible biomarker candidates in RA is relatively narrow [1]. The identification of RA biomarkers with applicability to early diagnosis and treatment stratification is therefore a relevant, unmet medical need [2].

There is preliminary evidence that sphingolipids might be relevant in the pathophysiology of RA [3–7]. A previous report described that the sphingolipid pathway regulating the intracellular levels of ceramide (Cer) and sphingosine-1-phosphate (S1P) is dysregulated in RA fibroblast-like synoviocytes [3]. In addition, sphingolipids have pleotropic pro-inflammatory effects through the induction of cyclooxygenase-2 and through genes encoding pro-inflammatory cytokines [4]. The membrane-bound glycosphingolipid GM3, moreover, is involved in the control of the activation of T cells in an animal model of arthritis [5] and of osteoclastogenesis in an animal model of multiple myeloma [6]. Additionally, the levels of lipid species, including sphingolipids, have been reported to be increased in the SF of RA and osteoarthritis patients [7]. Considering the aforementioned evidence, we hypothesized that the contribution of sphingolipids to inflammatory processes would likely be detectable in the serum of
RA patients and thus, that serum sphingolipids could eventually act as candidate biomarkers in RA.

Methods

Patient and sample selection

Eighty-two participants were included in the study. We collected information on age and gender from all participants. In addition, we collected information on age-of-onset, disease duration, DAS-28, RF, ACPA, HLA B27 and ongoing treatment of the patients by the time of the blood collection.

Participants were divided into five groups (Table 1, including demographic-, clinical- and medication data): untreated early RA patients (<1 year of disease duration) (n = 18); established active RA patients (>1 year after disease onset, DAS-28 > 3.2, under treatment with conventional disease-modifying antirheumatic drugs (cDMARDs) and corticoids (low-dose prednisolone <10mg/day)] (n = 19); untreated early arthritis patients not fulfilling the classification criteria for RA (<1 year after disease onset) (n = 13); established active SpA patients with peripheral symptoms (>1 year after disease onset, under NSAID treatment) (n = 12); and healthy controls (n = 20).

Patients were classified as RA if they fulfilled the ACR/EULAR 2010 classification criteria [8] and were classified as SpA if they fulfilled the ASAS 2011 classification criteria [9]. Exclusion criteria were applied accordingly.

Lipid measurements

We screened the serum levels of total Cer (as a sum of Cer 16:0, Cer 18:0, Cer 20:0, Cer 22:0, Cer 23:0, Cer 24:0, Cer 24:1, Cer 25:0 and Cer 26:0), total monohexosylceramide (HexCer; as a sum of HexCer 16:0, HexCer 16:1, HexCer 18:0, HexCer 20:0, HexCer 22:0, HexCer 23:0, HexCer 24:0 and HexCer 24:1) and sphingosine (So; d18:1) in 10 μL of serum by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) as previously described [10]. All samples were analysed in duplicate.

Statistics

We carried out multiple regressions on the serum lipid levels (Cer, HexCer and So; dependent variables) using two types of models, which were highly complementary.
As independent variables in the first type of models, we included each participant’s group (with the patient groups being coded categorically and no term being added for the control group), age at diagnosis and gender (coded as 0 or 1 for males and females, respectively), in addition to an intercept term and a variable that accounted for the fact that each sample was analysed twice (rep, coded as 0 or 1 for the first and second measurements, respectively).

In the second type of models, we included additionally the treatments with cDMARDs and corticoids as independent variables (both coded as 1 or 0, depending on whether a given patient was, or was not, on cDMARDs or corticoid medication, respectively). To facilitate the interpretation of the estimated coefficients, we normalized all variables so that their absolute values were always lower than, or equal to, 1. Our analyses did not explicitly consider disease duration or other clinical data because such information was intrinsically related to the grouping of the patients and/or unavailable for controls. Similarly, we did not consider NSAID treatment in our models, as only the SpA patients were continuously on this medication (Table 1). We applied Bonferroni correction for multiple comparisons within each type of analyses.

## Results

We found increased levels of So, HexCer and Cer in established RA patients vs controls, with the increase in So being the most significant [coefficient value (cv): 0.106, \( P < 0.001 \); Table 2, column A], followed by the increase of HexCer (cv: 0.077, \( P < 0.01 \); Table 2, column A), both of which remained significant after Bonferroni correction. Early RA patients, likewise, showed a significant increase in So vs controls (cv: 0.043, \( P = 0.042 \); Table 2, column A), although that increase did not survive Bonferroni correction. On the contrary, the established SpA group showed solely decreased levels of Cer compared to the control group (cv: -0.091, \( P = 0.005 \); Table 2, column A).

When additionally controlling for cDMARDs and corticoids, the increase of So remained significant in the early RA patients, although significance was lost after Bonferroni correction (cv: 0.043, \( P = 0.042 \); Table 2, column B). Besides, the increase of So lost statistical power in the established RA group (cv: 0.089, \( P = 0.342 \); Table 2, column B). In the established RA group, however, levels of HexCer remained significantly increased when compared to the control group (cv: 0.347, \( P = 0.008 \); Table 2, column B). Furthermore, like in the first model type, Cer levels remained significantly lower in SpA patients than in controls (cv: -0.093, \( P = 0.008 \); Table 2, column B). Still, no cut-off levels could be proposed that could be of interest for diagnosis.

In the second model type, age and corticoid therapy were also shown to be negatively associated with HexCer (Table 2, column B), but none of these findings survived Bonferroni correction. Notably, no significant difference was detected between the first and the second measurements of each lipid for any model (all \( P > 0.25 \)).

| Table 2 Serum lipid level alterations in the different patient groups compared with the control group |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Independent variables \ Model type** | **Dependent variables** | **Cer** | **HexCer** | **So** |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Intercept | 0.479*** | 0.483*** | 0.445*** | 0.469*** | 0.118** | 0.121** |
| Early non-RA | 0.029 | 0.028 | 0.074* | 0.067 | 0.043 | 0.042 |
| Established RA | 0.055** | 0.066 | 0.077** | 0.347** | 0.106*** | 0.089 |
| Early RA | -0.010 | -0.010 | -0.043 | -0.044 | 0.043* | 0.043* |
| Established SpA | -0.091** | -0.093** | 0.007 | 0.027 | 0.031 | 0.027 |
| Rep | 0.018 | 0.018 | 0.016 | 0.016 | 0.010 | 0.010 |
| Age | -0.005 | -0.011 | -0.128 | -0.161* | 0.096 | 0.091 |
| Gender | 0.012 | 0.013 | 0.004 | 0.010 | -0.014 | -0.013 |
| cDMARDs | - | 0.010 | - | -0.066 | - | 0.024 |
| Corticoids | - | -0.021 | - | -0.203* | - | -0.007 |

Results obtained, when controlling for the measurement number (1st, 2nd) of each lipid, age and gender, and either not controlling (columns A) or controlling (columns B) for both conventional disease-modifying antirheumatic drugs (cDMARDs) and corticoids effects. Effects of the independent variables on the lipid levels (dependent variables) are expressed by the corresponding estimated regression coefficients (cv), with positive (negative, respectively) coefficients meaning that higher values of the independent variables are associated with higher (lower, respectively) values of the lipid levels. All variables were scaled so that their maximum absolute value was equal to 1. Patients’ gender was coded with 0 (male) or 1 (female); ‘rep’ relates to the measurement number of the respective lipid (0 and 1 for the first and second measurements, respectively). *, ** and *** denote \( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \), respectively. Coefficients with \( P \)-values below \( <0.05/3 \) (i.e. surviving Bonferroni correction) are shown in bold. cDMARDs, conventional DMARDs; Cer, ceramide; HexCer, monohexosylceramide; So, sphingosine.

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Discussion

We observed an increase in the levels of certain sphingolipids in the serum of established RA patients compared to controls, with the increase in So having the highest magnitude and the increase in HexCer being the most robust, given that this increase was significant both when not controlling and when controlling for medication effects.

Taking into account that only the increase in the HexCer levels remained significant when controlling for medication, it is hard to conclude, at this moment, whether the other sphingolipid levels were increased due to disease-related aspects or rather due to medication effects. Previous studies on RA and other diseases [7, 11–14], however, suggest that the reported increases might have appeared due to disease progression; indeed, our second type of models suffered from lack of power due to the strong correlation between the patients’ group and their medication variables (see Table 1).

Sphingolipids have started to be identified as potential serum biomarkers in various diseases such as hepatitis related to HBV and HCV infection [11], Type I diabetes [12], lysosomal storage diseases [13], or Parkinson’s disease [14]. Less is known about sphingolipids in RA. A 3-fold increase in the Cer concentration and a 5.8-fold increase in the HexCer concentration were observed in the SF of RA patients when compared with controls [7]. In an arthritic rat model, serum Cer, HexCer, So and S1P were up-regulated, while sphingomyelins were down-regulated when compared with the respective control animal group [15]. In addition, higher activity of the serum secretory sphingomyelinase (S-SMase) enzyme was reported in patients with RA [16]. S-SMase is an extracellular member of sphingomyelinases, enzymes that hydrolyze sphingomyelin to Cer, and activation of SMases is pivotal for ceramide production during inflammation [16].

The influence of the combined therapy with cDMARDs and corticoids on sphingolipid serum levels is still unclear. MTX was found to decrease Cer-16 levels in cancer cell lines in vitro [17] and the influence of MTX on sphingolipids was reported in a metabolomics study in an arthritic murine model [18]. Notably, in the aforementioned study of sphingolipids in the SF of RA patients, only the treatment with intra articular corticoids was an exclusion criterion, so probably most patients were also on MTX and low-dose corticoids [7].

We found reduced levels of Cer in SpA patients, who were all on continuous treatment with NSAIDs. This observation relates with the findings of the above-mentioned study of sphingolipid profiling in RA rats, where treatment with the NSAID indomethacin reversed the increased levels of Cer and its metabolites to control levels [15].

The literature is very limited about sphingolipid level changes in the serum and brain during physiological aging in humans. Czubowicz et al. described that Cer increases, while S1P decreases, with age in the brain [19], and increased sphingomyelin levels were described with increased age in the Baltimore Longitudinal Study of Aging [20]. In our study, age had a negative association with HexCer, although this did not survive correction for multiple comparisons, which suggests the need for further studies.

In summary, we could detect significantly higher levels of sphingolipids (most notably HexCer) in the serum of established RA patients as compared with controls. This elevation is consistent with the known roles of sphingolipids in inflammation and is in agreement with previous findings in the SF of patients with RA, underlining the contribution of sphingolipids to the pathophysiology of RA. Nevertheless, as in early RA our findings were less consistent than those that we saw in established RA, the significance of serum sphingolipids as clinically useful biomarkers for RA remains unproven and its possible relationship with the cytokine environment of the different phases of the disease still has to be clarified.

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References


