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The potential value of blood biomarkers of intervertebral disk metabolism in the follow-up of patients with sciatica

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Abstract Study design: This is a prospective study with a follow-up period of 4 years. Objectives: The study aimed to evaluate the possible clinical utility of three biomarkers [i.e., keratan sulfate (KS), hyaluronan, and cartilage oligomeric matrix protein] measured in peripheral blood in severe acute sciatica at intake and follow-up. Summary of background: Our previous study and others have pointed out the interest of different laboratory tests in the acute phase of sciatica. Several blood biomarkers have been reported useful in the long-term follow-up of patients with osteoarthritis. We have found no information about the potential interest of these tests in spinal disorders. Methods: Patients were admitted to the hospital for intensive conservative management of acute sciatica ($n=82$). A subgroup of patients ($n=33$) was selected based on the duration of symptoms at visit 1, and included those with the shortest ($n=24$) as well as those with the longest ($n=9$) duration of sciatica. Blood samples were drawn, centrifuged, and the plasma frozen. Antigenic KS, hyaluronan, and cartilage oligomeric matrix protein were measured by ELISA. Patients were

re-evaluated at an average of 4.3 years (range: 2.1–6.8 years). Results: Thirty-three subjects with an average age of 49.2 ± 10.2 years participated. At intake, levels of the three biomarkers evaluated were within the range of normal values. No significant differences were found between the results of patients with a short history of sciatica (≤ 3 weeks) and those with a long duration of symptoms (> 20 weeks). At follow-up, a significant increase ($P < 0.05$) in all three biomarkers was found. Conclusions: A single measurement of these three biomarker molecules does not seem to have any diagnostic or therapeutic relevance in patients with acute radicular compression. The significance of the increase in all three biomarkers after a mean follow-up of 4.3 years is unclear; it might reflect metabolic processes involved in degenerative spinal disorders. Even though we found no correlation with clinical outcome, we believe that more research is needed.

Keywords Intervertebral disk · Keratan sulfate · Hyaluronan · Cartilage oligomeric matrix protein · Blood

Introduction

Sciatica of radicular origin still represents a significant clinical problem. As Bogduk recently reminded us, discogenic pain cannot be diagnosed clinically and is at best a supposition [4]. The prevalence of false positives in modern imaging techniques and the limits of clinical–radiological correlation are well known and contribute to making the task of clinicians more difficult [5]. Moreover, there are data suggesting that inflammatory and immunological phenomena [41] may play a role in radicular sciatica due to disc herniation.

For these reasons, biomarkers of metabolic changes in intervertebral disc disease have begun to attract the attention of researchers in the field. Recent studies have reported on the potential usefulness of measuring the concentrations of different substances in the cerebrospinal fluid (CSF) [7]. However, because of associated risks, collecting CSF is not, and will not be, a routine procedure in the diagnosis and treatment of patients with acute sciatica. From a clinical standpoint, the collection of peripheral blood samples would be a more suitable approach, provided levels of useful biomarkers could be detected. For example, serum pseudocholinesterase levels have been reported to be correlated with pain in patients with chronic spinal pain [8].

A majority of studies in the biomarker field have been carried out in patients with osteoarthritis, and as many as 14 different biomarkers have been explored [31]. Serum levels of keratan sulfate (KS), a glycosaminoglycan found at a high concentration in the major proteoglycan of cartilage, have been reported to be stable, fulfilling the requirements of a useful biomarker [22]. Because intervertebral disc tissues contain KS it is not surprising that serum levels of KS increase significantly after chemonucleolysis of a single lumbar disc [3, 29]. In addition, radiolabeled monoclonal antibodies against KS have been shown to detect *in vivo* intervertebral disk injuries [17].

The purpose of this study was to evaluate the levels, evolution and clinical utility of different blood biomarkers of disc and cartilage metabolism (1) during the acute phase of sciatica and (2) at a follow-up visit over 4 years later.

Our hypotheses at the beginning of the study were that duration of symptoms (leg pain) could be expected to influence selected biomarkers level at intake; and that a relationship may be found between the clinical outcome at follow-up and changes in the levels of selected blood biomarkers during the observation period.

Material and methods

This project was part of a prospective study of patients with acute severe sciatica of radicular origin who were admitted to the hospital for intensive conservative

management. The inclusion and exclusion criteria, the characteristics of the population and the methodology of the study have been previously described in detail [1], in summary, between 1993 and 1997, 82 patients with acute sciatica admitted to the hospital for intensive conservative care were included. They underwent five standardized evaluations at admission, discharge, and 3, 6, and 12 months after leaving the hospital. Blood samples were drawn at visit 1, in the prone position, after a night of bed rest. Heparin-coated tubes were used and the samples centrifuged to obtain plasma, which was distributed in 1-ml aliquots and kept frozen at -60°C until the biochemical analyses were performed.

At an average of 52.3 months after visit 1, a subgroup of patients was invited to attend a sixth evaluation performed by an independent observer according to the same protocol used for the previous five visits, in addition peripheral joint diseases were ruled out. As this evaluation was carried out on an out-patient basis, the appointments were scheduled at least 3–4 h after the subject got out of the bed to avoid the increase in biomarkers reported to occur during the first hour after arising from a night of sleep [27]. Patients were also specifically asked about any form of corticosteroid treatment as corticoid in large doses (>40 mg/day) may influence the KS value [39]. Two patients had received one injection of corticosteroids 3 and 9 weeks before the blood sampling, respectively, while a third one was on oral prednisone (5 mg/day). Moreover, a sub-sample of 14 out of these 33 patients underwent a second CT-scan that was limited to the intervertebral space where the initial lesion had been identified by the first imaging study performed at visit 1 [1].

At follow-up, a second blood sample was drawn, centrifuged and kept frozen. The plasma samples were sent to Rush University Medical Center (Chicago, IL, USA). In order to eliminate any laboratory bias, the samples were sent blinded, only labeled with numbers that did not give any information about the patients' identity or about the visit. The samples were analyzed by well-characterized ELISA techniques for three biomarkers: antigenic KS (AgKS) [38], hyaluronan (HA) [23], and cartilage oligomeric matrix protein (COMP) [40].

Patient population

Out of the total population of 82 patients hospitalized for severe sciatica, 33 patients were selected based on the criteria of duration of symptoms at visit one. The criteria included those with the shortest ($n=24$) as well as those with the longest ($n=9$) duration of sciatica at intake. Short duration was less than 3 weeks of severe pain and long duration was established as more than 8 weeks of pain. The mean duration of symptoms at intake was 12.3 days (ranging from 1 to 21 days) among patients

with the shortest duration of symptoms at visit one, hereafter designed as “short history.” The patients with a mean duration of symptoms of 140 days (ranging from 56 to 540 days) are hereafter designed as “long history.” We selected those subgroups of duration of pain to explore the potential diagnostic values of the selected biomarkers. The concept being that if no difference in values was found in the extreme durations (long and short history) little usefulness could be attributed to the group with intermediate duration.

Ethical committee

The regional ethical committee (Comité Intercantonal d’Ethique—Jura Fribourg Neuchâtel) approved the study (Approval number 12/1999).

Statistical analyses

Plasma levels of COMP, HA, and AgKS were measured using duplicate analysis: the mean value was used in all cases when performing statistical analyses. Repeated ANOVA was performed on the whole group ($n=33$) [except for AgKS, where $n=32$]. Repeated ANOVA (analysis of variance) was performed including duration and visits on the three biomarkers with and without log transformation.

The three biomarkers were checked for normal distribution using the Chi-square test and Kolmogorov–Smirnov test. The correlation matrix for COMP, HA, and AgKS was performed using Pearson correlation. Significance level was set at $P=0.05$ [43].

Results

Thirty-three subjects (9 women and 24 men) categorized by long and short history of sciatica pain duration took part in this study. Patient characteristics are summarized in Table 1. None of the clinical parameters measured were significantly different in the “short” and “long history” subgroups.

Disability measured by Oswestry Disability Inventory and pain (VAS leg pain) did not significantly correlate with any of the biomarkers over time (first and last visits); furthermore, no significant correlation with biomarkers was found in patients having had surgery at any time during the follow-up period with those not having surgery for sciatica.

In agreement with previously published studies [6, 12, 20, 25, 42], none of the patients with repeat imaging ($n=14$) showed a worsening of disc herniation in the final imaging studies [34].

Table 1 Characteristics of the population included in the long-term follow-up visit ($n=33$)

Gender (F:M)	9:24
Age in years [mean ($\pm$ SD)]	49.2 $\pm$ 10.2
Years of follow-up [mean; (range)]	4.3 (2.1–6.8)
Treated surgically (%)	45%
Low back pain (%)	51%
Low back pain by VAS ^a [mean; (range)]	23.5 (1–54)
Leg pain (%)	24%
Leg pain by VAS ^a [mean; (range)]	32.2 (10–86)
Oswestry Disability Inventory [mean; (range)]	6.7% (0–57%)
Full or partial disability pension	25%

^a These values represent only the subjects reporting current pain, low back pain (51%) and leg pain (24%)

For antigenic KS, the intra-assay variation was less than 3% and the inter-assay variation was less than 4%. For HA, the intra-assay variation was less than 4% and the inter-assay variation was less than 6%. For COMP the intra-assay and inter-assay variation did not exceed 10%.

The results of the biomarkers are summarized in Table 2. A large majority of patients exhibited increases in the concentration of one or more biomarkers during the follow-up period. Limiting the definition of a significant increase in biomarker level at follow-up to those reaching $\geq 100\%$ of the initial value (i.e., more than doubling), 33% of patients fulfilled this definition for AgKS, 21% for HA, and 27% for COMP. Several patients exhibited greater than 100% increases in levels of two or more markers: AgKS and COMP (five patients), AgKS and HA (three patients), HA and COMP (two patients), and finally, all three biomarkers (two patients).

The corollary is that only a minority of patients presented decreased levels of biomarkers at follow-up. Actually, none of the subjects exhibited a reduction from initial plasma levels (first visit) of AgKS, while ten cases (33%) showed decreased plasma levels of HA, and four (12%) showed decreased plasma levels of COMP.

Plasma levels of the three biomarkers were significantly correlated, with coefficients of 0.40 between AgKS and HA, of 0.53 between AgKS and COMP, and 0.60 between HA and COMP.

Normality testing showed a Gaussian distribution for all three biomarkers. Repeated ANOVA of clinical parameters, including visits, short and long duration groups, and visits within duration groups showed no significant effects, indicating that there is no difference between the short- and long-history groups in specific marker values. The results were similar for data analyzed with and without log transformation (Table 3). The results, while interesting, suggest that measurement of these markers in this clinical population probably have limited clinical value.

Table 2 Blood levels of biomarkers expressed as means and (standard deviation): antigenic keratan sulfate (KS), hyaluronan (HA), and cartilage oligomeric matrix protein (COMP)

	AgKS (ng/ml) (visit 1)	AgKS (ng/ml) (follow-up visit)	HA (ng/ml) (visit 1)	HA (ng/ml) (follow-up visit)	COMP (ng/ml) (visit 1)	COMP (ng/ml) (follow-up visit)
“Short” history $n=24$	251 (87)	396 (128)	15 (11)	18 (14)	1,471 (543)	2,431 (1,291)
“Long” history $n=9$	269 (68)	438 (123)	27 (20)	26 (20)	1,414 (590)	1,848 (692)
Total population $N=33$	273 (125)	422 (144)	18 (15)	20 (16)	1,455 (548)	2,272 (1,178)

“Short” history designates patients who were admitted to hospital with a mean duration of sciatica of 12.3 days. “Long” history designates the subgroup of patients who were admitted with a mean duration of pain of >140 days

Table 3 Repeated ANOVA for visits (first and last visit) and groups (long $n=9$ and short duration $n=24$) for three biomarkers (antigenic keratan sulfate (AgKS), hyaluronan (HA), and cartilage oligomeric matrix protein (COMP) with and without log transformation

	Without log transformation			With log transformation		
	Visits	Groups	Visits $\times$ groups	Visits	Groups	Visits $\times$ groups
AgKS (P)	55.10 (0.0001)	0.04 (0.8414)	0.56 (0.4598)	60.13 (0.0001)	0.27 (0.6046)	0.14 (0.7103)
HA (P)	0.17 (0.6827)	4.06 (0.0525)	0.61 (0.4417)	0.27 (0.6088)	4.56 (0.0408)	0.04 (0.8338)
COMP (P)	11.00 (0.0023)	1.23 (0.2763)	1.56 (0.2204)	23.40 (0.0001)	1.18 (0.2853)	1.07 (0.3085)

Numbers in bold indicate significance ($P<0.05$)

Discussion

The fact that the plasma levels of each biomarker showed a very significant rise at the time of the follow-up visit, 4.3 years after the time of the first analysis, is an unexpected and surprising finding. While we are unable to provide a simple explanation for the increase in the levels of the biomarkers, we were able to rule out certain possibilities. Samples from the two visits were stored frozen and analyzed on the same day. Because the antigenicity of at least one of the markers (i.e., AgKS) does not decrease over years of storage in a freezer and is not affected by repeated freezing and thawing [38], we do not believe that technical problems could have contributed significantly to the increase. As far as we were able to determine, there was no measurable difference in (a) the time venipuncture was performed and (b) the level of physical activity of the patients at the time of the two visits. Interestingly, neither of these potential variables would have been expected to cause levels to vary by more than 10% [27]. We also have considered the fact that samples were shipped on two different occasions. In the first shipment, the samples were clearly labeled allowing the laboratory to distinguish the first and the second samples from each patient. However, the similarity between the means of the two samples in the short and long history subgroups, despite the fact that the tests were performed 1 year apart, is a strong argument against our technical concerns.

It is widely accepted that because greater than 95% of AgKS is found in cartilage aggrecan [22], the level of AgKS in blood provides an indirect measure of the metabolism of this proteoglycan in cartilaginous

structures, including intervertebral discs [9]. The values for plasma levels of AgKS of our patients were within the range of patients with sciatica described in the literature. For example, in a study by Muralikuttan et al., patients with disk herniation had a mean AgKS blood level of 214 ± 22 ng/ml before either surgery or chemonucleolysis [29]. Block et al. have reported similar values, 254 ± 148 ng/ml, among patients undergoing chemonucleolysis and 192 ± 58 ng/ml among those referred to surgery [3]. All these values are within the range of the values reported for normal subjects: 257 ± 41 [3]; 251 ± 78 [38]; 261 ± 51 [37] ng/ml. According to these data from the literature, at the first visit, our patients exhibited normal blood levels of AgKS. The role of KS as a biomarker of mechanical loading of the spine was reviewed by Kuiper et al., who concluded that “the serum level of KS has the potential to be a biomarker of degeneration of intervertebral discs” [22]. Mehraban et al., presenting the results of their study of patients with osteoarthritis and rheumatoid arthritis, also concluded that serum AgKS increases in the presence of cartilage degradation, but suggested the elevations do not quantitatively define the extent or duration of articular disorder [28]. The results of our longitudinal exploratory study of KS levels in the blood of patients with sciatica are compatible with the contention raised by Kuiper’s group but this hypothesis clearly needs further testing.

Hyaluronan, unlike AgKS, is found at high concentrations in many different tissues within the body [31]. In synovial joints, HA is synthesized by the B synoviocytes and released into the joint space [26]. It has also been suggested that the dermis could be a source of the HA

found in the blood [27]. In a dog model of osteoarthritis, a sustained increase in the serum level of HA has been reported and attributed to an increased rate of synthesis by synovium [26]. Serum levels of HA significantly increased during the first hour of activity after a night of bed rest, both in patients with RA and controls [27]. In a recent prospective study, HA blood levels were significantly related to age [30]. Among patients with knee osteoarthritis, serum HA levels could predict disease outcome [35]; these results were confirmed by others [13]. It has recently been reported that the human nucleus pulposus contains more HA in the case of an acute disc herniation than in the case of older degenerative disc modifications [44]. We found no study assessing blood levels of HA in patients with spinal disorders. Notwithstanding this, it was a little surprising that the changes in levels of AgKS and HA were strongly correlated [27].

Cartilage oligomeric matrix protein is found predominantly in cartilage, but also in tendon and synovium [10]. In a 5-year prospective study including 81 patients with knee osteoarthritis, baseline serum levels of COMP did not differ between progressive and non-progressive osteoarthritis. However, the levels increased significantly during the first year in progressive cases [36]. Kühne et al. suggested that a subgroup of patients with elevated and increasing levels of COMP were at risk for developing post-traumatic osteoarthritis of the knee [21]. In a 1-year prospective study of 48 patients with osteoarthritis of the hip, the baseline COMP concentrations correlated with the baseline joint space width and with its mean yearly reduction. Therefore, this marker could be “of interest” for the detection of patients with rapidly progressing hip osteoarthritis [11]. In a larger study, serum COMP levels were significantly higher among subjects greater than 65 years of age compared with those aged 45–64 years. Moreover, the COMP serum levels of the osteoarthritis group were significantly higher than those of the control group. Finally, there was a significant increase in COMP serum levels with severity and number of joints involved [10]. These authors considered two hypotheses to explain their results, i.e., an increased release due to destabilization of the matrix or an increased synthesis [10].

In a 1-year prospective study including 39 osteoarthritis patients, using 14 molecular markers, the best discrimination between patients and controls was obtained by means of a cluster including tumor necrosis factor (TNF) receptor type II, COMP, and aggrecan fetal epitope [30]. In the second part of this study, the clinical outcome at 12 months was not correlated with any of the biomarkers [31]. This finding demonstrates the difficulties one encounters in understanding such a complex problem as osteoarthritis [33].

Finally, in a recent study of 24 rheumatoid arthritis patients followed for 5 years, COMP was unrelated with other markers as well as with joint damage progression

[32]. We found no study assessing blood levels of COMP in patients with spinal disorders.

Development of osteoarthritis of the spine

It has been stated that in humans, the proteoglycan content of intervertebral discs decreases with age because of a decreased synthesis and/or increased release [18, 24]. The results of a study with cells from the rabbit annulus were in agreement with these statements [24]. However, longitudinal studies in humans indicate that our period of follow-up (4.3 years) should not explain the differences in biomarkers found between the two blood samples [9].

A recent autopsy study showed that specimens from individuals before the age of 40 years might show microscopic degenerative changes in the discs and facet joints. In the same study, no significant correlation was found between the grades of degeneration for the discs and the associated facet joints assessed separately at either level [16]. A recent study showed that in the general population, there is a strong genetic effect for disc degeneration of the spine [2]. Aggrecan gene polymorphism has been found to be associated with multilevel disc degeneration [19].

Limitations

The design of our study does not allow us to know from which tissue the biomarkers studied are released. A control group or a longitudinal design would have shed some light on the results; however, we did not have the possibility to do that in this exploratory study. One of the difficulties using a control group for these biomarkers is that the range of normal values is very large in the general population for at least KS and somewhat less for COMP and HA. There is also a large prevalence of asymptomatic disc herniation in the general population [5].

Furthermore, the focus of this exploratory study was to evaluate the relationship between selected biomarkers and clinical outcomes. For an exploratory study, it is reasonable to start looking for possible answers within a disease group even if it can be considered as a methodological limitation.

Conclusions

At intake, patients with acute severe sciatica showed normal levels of three biomarkers (AgKS, HA, COMP), therefore the clinician cannot rely on such tests for decision making. At 4.3 years follow-up, we found a significant increase in serum levels of AgKS, HA, and

COMP. Demographic data, duration of symptoms, clinical outcome, or having undergone surgery, were not correlated with any of the biomarkers.

According to the evolution of these markers in osteoarthritis of peripheral joints, a possible explanation is that our patients were progressively developing spinal osteoarthritis. It has been reported that degeneration of the disc is a probable pre-requisite for disk herniation

[14, 15]. Our results could suggest that in the whole process of disc degeneration, acute sciatica is only the tip of the iceberg. Perhaps the complexity reported for osteoarthritis [33] also applies to degenerative lumbar disc disorders. A prospective study with long follow-up designed to evaluate the incidence and a quantitative evaluation of degenerative spinal disorders could be useful to better understand the meaning of our findings.

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