Cartilage Oligomeric Matrix Protein as a Marker of Osteoarthritis

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This report will address the utility of cartilage oligomeric matrix protein (COMP) as a biomarker of osteoarthritis (OA). I will discuss what we have learned from our work with the Johnston County Osteoarthritis Project.

How would we like to use a biomarker? We would like it to identify OA even before radiographic changes develop. We would like better ways to diagnose OA and to provide a prognosis. For clinical trials, we would like to be able to use a biomarker to identify at the outset subjects at high risk of progression. This would permit us to reduce the sample size and/or shorten the duration of the study. If a biomarker were able to do this and thereby let us avoid the need to treat subjects with active drug or placebo for 2 or 3 years until a difference between treatment groups becomes apparent with respect to radiographic change, this would be a good thing. All this would require, however, a marker with high sensitivity and very high specificity. We are probably not there yet.

Also, we will need standardized markers. Much of the data I will discuss was obtained using a variety of assays, performed with both monoclonal and polyclonal antibodies. Finally, we will need normative data. What are the levels of the marker in age and sex matched nonarthritic individuals?

COMP, also known as thrombospondin 5, is a 524 kDa glycoprotein. It is a member of the thrombospondin family of extracellular calcium-binding proteins, which appear to be important in assembly of the extracellular matrix and matrix-matrix protein interactions. COMP is composed of 5 identical subunits. The carboxyterminal globular domain binds to collagens I, II, and IX. COMP plays some role in the organization of collagen fibrils. At their aminoterminal ends, the 5 strands of the molecule come together in a coiled–coil domain that appears to play a role in the storage and delivery of hydrophobic cell-signaling molecules, such as vitamin D (Figure 1). Although COMP was initially felt to be cartilage-specific, over the past few years it has been identified in all structures of the joint, including ligaments, meniscus, tendons, and synovium. It is found also in osteoblasts and vascular smooth muscle. In animal studies, its distribution is even broader.

The primary sequence of rat, mouse, and human COMP is known. Mutations in the type 3 binding repeats and in the carboxyterminal globular domain (Figure 1) lead to pseudoachondroplasia and other epiphyseal dysplasias[1]. I will review our work with monoclonal antibody (mAb) directed against 17-C10 as well as our newest studies, which have been performed with a sandwich ELISA, using antibodies 16-F12 and 17-C10 (Figure 1).

COMP has been thought of as a marker of cartilage catabolism. Serum levels of COMP are elevated in patients with OA, in comparison to healthy subjects[1]. Longitudinal studies of patients with knee OA[2,3] have found that rising levels of COMP correlate with progression of disease. Among people with chronic knee pain, those who developed incident radiographic disease at followup 3 years later had higher COMP levels at baseline[3] than those who did not. Notably, baseline measurements of COMP in those studies were not very useful as a predictor of OA progression in those who had radiographic OA at baseline. However, in a study by Conrozier, *et al*[4], elevation of the serum COMP level at baseline predicted progression of symptomatic hip OA. Recently, elevations of serum COMP have been found in patients with chondromalacia patellae[5].

In all these studies, COMP levels were determined with the use of polyclonal antibodies. However, Vilim, *et al*[6] have produced several mAb. We have utilized his mAb, 17-C10, which recognizes a conformational epitope in the domain of the epidermal growth factor-like repeats near the center of the COMP subunit (Figure 1) in a cross-sectional analysis, in which higher serum COMP levels were found to be associated with the presence of radiographic knee OA and other measures of radiographic severity[7]. Vilim[8] has also demonstrated an association between COMP levels and synovitis, a finding that is not surprising insofar as COMP is present in synovium as well as in cartilage.

The new sandwich ELISA we currently use recognizes 2 domains — 16-F12 in the amino terminus and 17-C10, which resides within the EGF-like domain. Using this assay, Vilim[9] found that baseline COMP measurements predicted the progression of radiographic knee OA, and we have demonstrated ethnic and sex differences in COMP levels[10].

What have we learned about COMP from the Johnston County Osteoarthritis Project? We recruited a population-based cohort of 3200 African-Americans and Caucasians living in a relatively rural setting in North Carolina, which,

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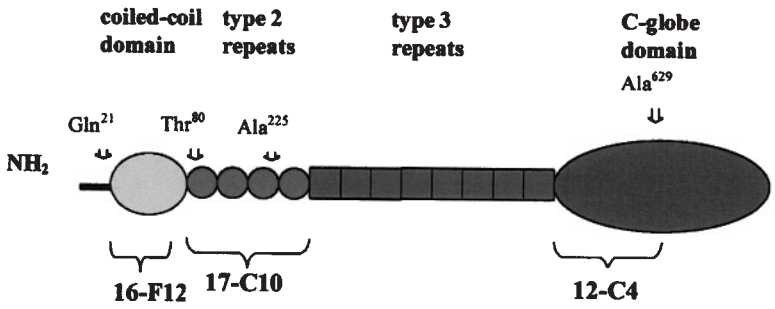

Figure 1. Structure of COMP. Monoclonal antibodies 17-C10, 16-F12, and F12-C4 recognize the regions of the molecule depicted. With permission from Vilim, *et al*. Clin Chim Acta 2003;328:59-69.

however, is becoming increasingly urbanized. At baseline, participants were at least 45 years of age. These data were obtained at the baseline examination, during which antero-posterior (AP) radiographs of both knees and of the pelvis were obtained and a joint examination was performed. For our biomarkers substudy, we performed a stratified simple random sampling to achieve balance, depending upon whether the subject had OA or did not and upon ethnic group. We aimed for a 50% ratio of men to women, evenly divided among the age groups of 45–54, 55–64, and ≥ 65 years.

We considered subjects to be "OA affected" if the Kellgren-Lawrence (K-L) radiographic grade of OA severity was ≥ 2 in at least one knee. We were particularly interested in having a control group that was as "clean" as possible. Most studies of this type have examined subjects with either knee OA or hip OA. However, because we had radiographs of both joint sites, we required that our control subjects had a K-L grade of 0 in both knees and both hips.

We used mAb 17-C10 to describe the distribution of serum COMP levels in a large, radiographically defined, normal Caucasian population[7]. We wanted to ascertain whether this would be helpful in studying OA-affected and OA-unaffected individuals in a population-based, as opposed to a patient-based, sample.

We used similar outcome measures for each of our analyses: the primary outcome was the natural logarithm (ln) of the serum COMP concentration. Like many other biomarkers, COMP is not normally distributed. However, with the transformation we performed, we were able to obtain a normal distribution of serum ln COMP. We performed an analysis of covariance in which we examined the effects of age, sex, and body mass index (BMI). Because we were interested also in whether COMP levels were affected by the "amount" of OA, we looked at this in relation to the unilaterality or bilaterality of knee OA, the number of knees and hips exhibiting radiographic OA, and the radiographic severity of OA in both knees and both hips.

As shown by the p values derived from the log-transformed data, COMP levels in our sample of 291 subjects were higher among subjects 65–85 years of age than in younger individuals (Table 1). Further, across the entire age spectrum, people with OA had higher COMP levels than those who did not have OA (Figure 2). As shown in Table 1, we found no real difference in COMP levels between men and women and not much of an effect of obesity[7]. In men, the association of COMP level with age was less striking than in women.

As shown in Figure 3, after adjustment for age, sex, and BMI, serum COMP levels increased with worsening radiographic severity. When we examined the level of COMP in relation to the unilaterality or bilaterality of knee OA or the number of affected joint sites (hip and knees, possible range = 0–4) we found that the COMP level increased significantly with the number of joints affected (Figure 4)[7].

We were also interested in knowing whether a relationship exists between COMP and joint pain, especially in subjects who do not have radiographic evidence of hip or knee OA[11]. We focused on a group of patients whose radiographs were negative for changes of OA but who had a positive response to the question: "On most days, do you have pain, aching or stiffness in your right or left knee?" Some of these subjects had clinical signs of disease (e.g., bony enlargement, crepitus, effusion, bony or soft tissue tenderness), but none had radiographic changes of OA. The

Table 1. Mean serum cartilage oligomeric matrix protein (COMP) levels by demographic and clinical variables. With permission from Clark, *et al*. Arthritis Rheum 1999;42:2356-64.

Group	n	COMP, ng/ml ± SD	p*
Age 45–54	98	1058.1 ± 432.4	
Age 55–64	95	1038.6 ± 313.3	0.0001
Age 65–85	98	1302.1 ± 496.7	
Men	147	1160.3 ± 442.2	
Women	144	1107.0 ± 432.5	0.2773
BMI ≤ 30	189	1098.1 ± 402.2	
BMI > 30	102	1200.4 ± 491.5	0.0879

* p values for overall linear trend, calculated using ln COMP values; p < 0.01 for pairwise comparisons of the age 65–85 group with each of the 2 younger age groups.

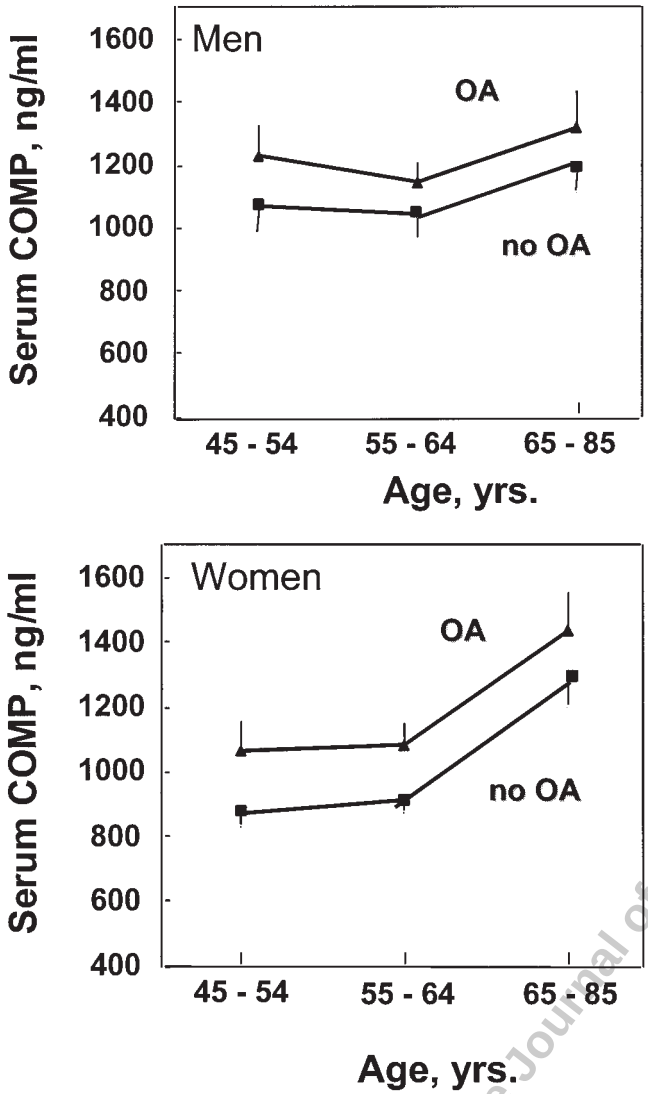

Figure 2. COMP levels were higher in subjects with OA than in those who did not have OA. A: men, B: women. With permission, from Clark, *et al*. Arthritis Rheum 1999;42:2356-64.

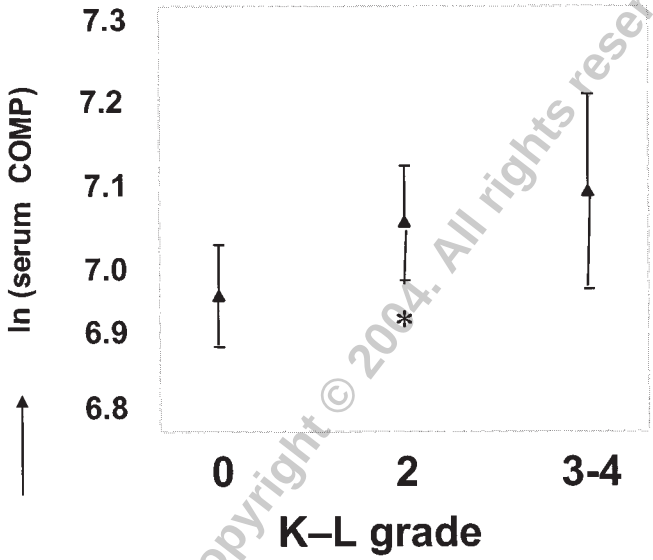

Figure 3. Serum COMP concentration, in relation to radiographic severity of OA, based on the K-L grade. Results are adjusted for age, sex, and BMI. Data represent mean values ± 95% confidence intervals. *p < 0.05, compared to subjects with K-L grade 0. With permission from Clark AG, Jordan AM, Vilim V, et al. Arthritis Rheum 1999;42:2356-64.

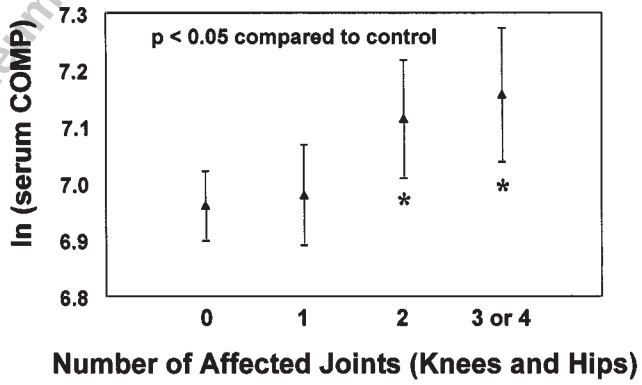

Figure 4. Serum COMP concentration in relation to the number of hip and knee joints affected. Data are adjusted for age, gender, and BMI. Data points represent mean values ± 95% confidence intervals. *p < 0.05, compared to subjects with no hip or knee OA. With permission from Clark, *et al*. Arthritis Rheum 1999;42:2356-64.

question we asked to elicit symptoms of hip OA was identical to that for the knee. In addition, however, we asked about groin pain as well as hip pain and, on physical examination, we evaluated pain on internal rotation of the hip.

The models we ran took into account symptoms, signs, the examiner's global assessment, and whether the subject fulfilled American College of Rheumatology (ACR) clinical criteria for hip or knee OA. Our subjects were, on average, about 60 years old with a mean BMI of about 27, and their COMP levels ranged from 292 to ~2300 ng/ml (median 959 ng/ml).

We found that the presence of hip symptoms, as defined above, was associated with a significantly higher COMP level than the absence of hip symptoms, although no such difference was seen with respect to knee symptoms (Table 2). Similarly, serum ln COMP was associated with meeting ACR clinical criteria for hip OA, but not for knee OA[11].

It is important to note that the above results were all derived from cross-sectional analyses. We have now nearly completed our first set of followup examinations and will have an opportunity to look at these relationships longitudinally.

In summary, among subjects with no radiographic evidence of either hip or knee OA, those with clinical signs or symptoms of hip OA or those fulfilling ACR criteria for a clinical diagnosis of hip OA were more likely to have a higher COMP level than those without such hip signs or

Table 2. Serum Ln COMP levels in relation to pain in hip or knee in the absence of radiographic hip or knee OA*. Adapted from Dragomir, *et al*. Osteoarthritis Cartilage 2002;10:687-91.

	Median COMP Level, ng/ml (1st quartile–3rd quartile)	Mean Difference, Ln COMP adjusted means (95% CI)	Adjusted p
Hip symptoms			
No	919 (771–1,108)	0.123	0.046
Yes	1,104 (863–1,415)	(0.002, 0.244)	
Knee symptoms			
No	951 (810–1,231)	–0.058	0.345
Yes	1,003 (817–1,340)	(–0.178, 0.063)	

* From linear model for ln COMP containing variables for symptoms in knees, hips, shoulders, upper/mid-back and elbows; age; gender; and body mass index.

symptoms. In contrast, COMP levels did not help differentiate subjects with knee signs or symptoms from those without these clinical features. Thus, serum COMP levels may be a useful marker of pre-radiographic hip pathology[11]. We will look closely at this group of individuals to determine whether they proceed to develop radiographic OA or worsening of their symptoms.

Finally, I present data on ethnic and sex differences in serum levels of COMP, measured by sandwich ELISA[6,10]. As in our previous analyses, we used the definitions of OA stated above and examined the serum ln of COMP in relation to age, sex, BMI, weight and height, and the presence of other symptomatic joints.

In a sample of 379 African-Americans and 309 Caucasians, the African-Americans had a higher BMI than the Caucasians, and the African-American sample contained a slightly higher proportion of women. As shown in Figure 5, among both African-American and Caucasian controls and subjects with OA, age was associated with the serum COMP level. Caucasian men with OA had a higher serum COMP level than those without OA. The relationship was similar in African-American men, although not as dramatic.

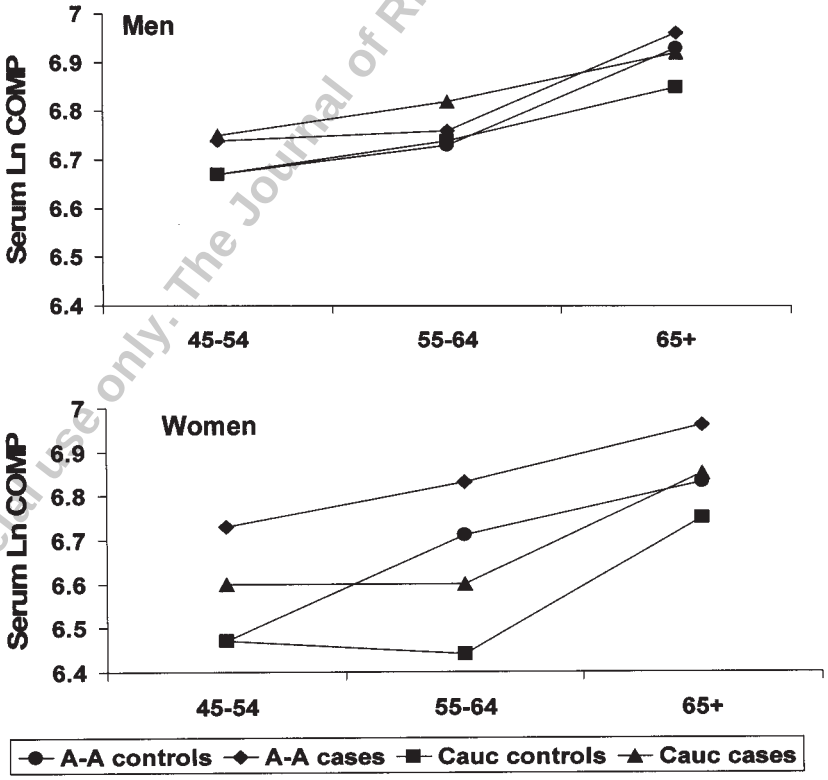

Figure 5. Mean serum COMP levels in men and women in relation to presence or absence of radiographic knee OA, ethnicity, and age group. A–A: African-American; Cauc: Caucasian. With permission, from Jordan, *et al*. Arthritis Rheum 2003;48:675-81.

Further, there was not much difference between African-American and Caucasian men with respect to mean serum COMP levels. However, regardless of status of their OA, African-American women had significantly higher levels of COMP than Caucasian women[10].

We confirmed that COMP levels were associated with age, as noted previously. The ethnic and sex differences in serum COMP could not be explained, however, by differences in age, BMI, height, the presence or severity of radiographic OA, or the presence of other symptomatic joints.

In developing standards for serum COMP and in collecting normative data, it will be important to take age, sex, and ethnicity into account. In addition, postmenopausal women who receive hormone replacement therapy have lower COMP levels than women who do not[12]. Despite these variables, COMP may be an important biomarker of OA. Although it is unlikely to be useful as a solitary test, the possibility that COMP may be useful in combination with other biomarkers or other demographic data is worthy of further study.

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