Correlation of Acoustic Velocity of Synovial Fluid with Markers of Inflammation in Arthritic Patients

Yeong-Jian Jan Wu, Tsung-Tsong Wu,¹ Yeong-Huei Liu,¹ Huei-Huang Ho, and Shue-Fen Luo

Abstract: Analysis of synovial fluid is important in the evaluation and treatment of arthritic conditions. This study measured the acoustic velocity of synovial fluids in patients with degenerative joint diseases, crystal arthropathy, and other inflammatory arthropathies using the ultrasonic pulse-echo method. The measured acoustic velocities of these bio-fluids were then correlated with clinical parameters including the synovial white blood cell count (WBC), clarity, viscosity, string test, erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP). Results showed that the acoustic velocities were correlated with ESR \( p = 0.0016 \) and CRP \( p = 0.0001 \). The mean acoustic velocity of inflammatory synovial fluids, defined as synovial fluids with WBCs of more than 2000/mm\(^3\), was greater than that of synovial fluids with WBCs of less than 2000/mm\(^3\) \( (1550 \pm 4.5 \text{ m/s} \text{ vs } 1544 \pm 1.5 \text{ m/s}, p = 0.007) \). This study demonstrated that the acoustic velocity of synovial fluid correlates well with severity of inflammation. These findings suggest that measurement of acoustic velocity may be useful in the clinical evaluation and management of arthritic conditions.

Materials and Methods

Synovial fluid is a clear, sticky liquid with an appearance similar to egg white. It is a dialysate of blood plasma into which hyaluronate is secreted by the synovial lining cells (synoviocytes). Normally, the amount of synovial fluid present in the joint space is small, with large joints such as the knee containing up to 4 mL of synovial fluid [1]. In arthritic conditions, the amount of joint fluid is often increased, and the fluid can be aspirated for analysis.

The quantitative measurement of synovial fluid requires a relatively fresh specimen so that the white blood cell count (WBC) can be determined [2, 3]. However, spontaneous clotting and clumping of leukocytes can occur in aspirated synovial fluids despite prompt transport, making cell count impossible. A study of the acoustic wave speed in synovial tissue showed that the wave speed increased with the amount of connective tissue [4]. The acoustic properties of bio-fluids have been evaluated by acoustic microscopy and the wave speed has been shown to correlate well with the amounts of various proteins in urine and of hemoglobin in blood samples [5, 6].

In the present study, acoustic velocities of synovial fluid samples from patients with various arthritic conditions were measured and correlated with clinical markers including erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), and synovial WBC, clarity, viscosity, and string test.

Chang Gung Memorial Hospital Rheumatology service, Tao-Yuan, during the period from January to June 1997. Fluid samples were collected in test tubes containing sodium heparin for routine synovial fluid analysis. Eleven patients had rheumatoid arthritis (RA), eight had spondyloarthritis (SSA), 10 had gout, one had calcium pyrophosphate deposition disease (CPPD), three had degenerative joint disease (DJD), and two had septic arthritis. The specimens were evaluated for monosodium urate monohydrate (MSUM) and calcium pyrophosphate dihydrate crystals using a polarized microscope (Olympus BH2, Tokyo, Japan). Samples were also evaluated by alizarin red S stain to screen for crystals that contained calcium or phosphate, such as hydroxyapatite [7].

WBC and its differential in synovial fluid samples were determined if possible. WBC was used to classify synovial fluid samples into normal (<200/µL), group I (non-inflammatory, 200–2,000/µL), group II (inflammatory, 2,000–100,000/µL), and group III (purulent, >50,000/µL, usually >100,000/µL), as described by Gatter and Schumacher [8]. The synovial fluid viscosity, clarity, and string tests were also determined according to methods described by Gatter and Schumacher [8]. Viscosity of synovial fluid was determined and recorded as normal, low, or variable. Clinically, low viscosity is associated with inflammatory synovial fluid [1, 8]. Clarity was determined by visualization, and described as transparent, translucent, or opaque. Translucent or opaque synovial fluid is associated with inflammatory arthritis [1, 8]. The string test result was recorded as more than 5 cm or less than 5 cm, with more than 5 cm indicating normal viscosity.

Blood inflammatory markers such as CRP and ESR were determined if possible. Westergren ESR values of 0 to 15 mm/hour for males and 0 to 20 mm/hour for females were defined as normal. A CRP of less than 5 mg/dL was defined as normal.

A suspension of synthetic hydroxyapatite (calcium phosphate hydroxide type 1) crystals in 0.001 M phosphate buffer pH 6.8, 4.12 g wet weight (Sigma Chemicals, St Louis, MO, USA) were diluted in normal saline into 1:6, 1:12, 1:24, 1:48, and 1:96 samples. Calcium phosphate (neutral brushite) crystalline suspension in water, weight 20 g, solid content 26% (Sigma Chemicals), was diluted to 1:4, 1:8, 1:16, 1:32, and 1:64 samples in normal saline. Ultrasonic measurements were made at a controlled temperature of 21°C, and six measurements were made for each sample.

Acoustic velocities of synovial fluids were measured at room temperature using the ultrasonic pulse-echo method, with the experimental set-up shown in Fig. 1. The amount of synovial fluid needed for the measurement was 1 mL. In the measurement, an ultrasonic pulse was sent out from an ultrasonic probe via a 5-MHz transducer. The same transducer then received the echo reflected from the bottom of the fluid sample container. The time difference between the successive echoes is the round-trip travel time of the ultrasonic pulse from the transducer to the bottom of the container. However, because the precise distance between the transducer and the bottom of the container is relatively difficult to determine, the ultrasonic transducer was fixed on a holder with a precision micrometer controlled stage to avoid the need to measure this distance. At an initial reference position, a round-trip travel time was measured first as t1. The transducer was then moved toward the bottom of the container by a small distance, d, and the second round-trip time t2 was measured. The acoustic velocity of the sample fluid was then determined as v = 2d(t2−t1).

Since the acoustic velocity is temperature dependent, fluid samples were allowed to stand at room temperature for half an hour, and the corresponding room temperature at the velocity measurement was recorded (20.3°C–24.5°C). The measured acoustic velocities were then calibrated to 35°C based on the calibration curve of water.

Statistical Package for Social Science (SPSS Inc, Chicago, IL, USA) linear regression analysis was used to examine the relationships between acoustic velocity and synovial WBC, ESR, and serum CRP. The correlation coefficient r was calculated. Two-tailed t-test was used to analyse the acoustic velocity correlation with subgroups of synovial fluid WBC, viscosity, clarity, string test, and serum CRP. A p value of less than 0.05 was considered statistically significant.

### Results

There were 26 samples for which the WBC was measurable, while the remaining samples showed synovial fluid clotting despite collection in a heparinized tube. Of the 26 samples, nine were from RA patients, three from SSA, nine from gout, three from DJD, and two from septic arthritis. Samples with WBCs of less than 2000/mm³ had a mean acoustic velocity of 1,544.0 ± 1.5 m/s. Except for a sample that had a WBC of 100/mm³, the red blood cell count of 73,300/mm³ (which appeared to be a traumatic aspiration) had an acoustic velocity of 1,546.4 m/s, while the rest had acoustic velocities between 1,542.3 and 1,543.9 m/s. The acoustic velocity of synovial fluids with a WBC of less than 2000/mm³ was significantly lower than that of fluids with a WBC of more than 2000/mm³ (p = 0.007; Table). The
Acoustic Velocities of Synovial Fluid

The highest velocity measured was 1557.7 m/s in a sample from an SSA patient with a WBC of 36,900/mm³. There was a general trend (r = 0.30) that the higher the WBC, the greater the wave velocity. When samples were subdivided into gout and other groups, this correlation was better (r = 0.53) for the gout group than for the whole group.

There were 24 samples (9 RA, 5 SSA, 5 gout, 1 CPPD, 1 septic, and 3 DJD) with ESR data. A higher ESR in the blood of arthritis patients was correlated with a greater acoustic velocity (r = 0.61, p = 0.0016).

There were 22 samples (9 RA, 7 SSA, 2 gout, 3 DJD, and 1 CPPD) with CRP data. A higher serum CRP was associated with a greater acoustic velocity (r = 0.73, p = 0.0001). For CRP concentrations of greater than 20 mg/dL, the acoustic velocity was greater than 1547 m/s in 10 of 11 patients. When samples were grouped into those with CRP concentrations of less than 5 mg/dL (non-inflammatory) or more than 5 mg/dL (inflammatory), the difference in acoustic velocity was not significant. The association of acoustic velocities of synovial fluids with different parameters including WBC, CRP, clarity, string test, and viscosity are summarized in the Table.

Acoustic velocities of synovial fluids increased with an increase in synthetic crystals from hydroxyapatite and calcium phosphate neutral brushite (Fig. 2). Normal saline had a mean acoustic velocity of 1,499.6 m/s.

### Discussion

Routine synovial fluid analysis includes gross examination of color, viscosity, and turbidity. The viscosity reflects the amount of hyaluronic acid in the fluid, while the turbidity reflects the number of cells in the fluid [9]. A non-inflammatory (group I) synovial fluid is characterized by high viscosity, straw color, and transparent clarity. An inflammatory (group II) synovial fluid is characterized by low viscosity, straw to opalescent color, and translucent or opaque clarity. In septic arthritis (group III), synovial fluid is characterized by variable viscosity, variable color, and opaque clarity [8]. While these qualitative descriptions can be made immediately upon aspiration of joint fluid, such assessments are inherently subjective.

Quantitative analysis of synovial fluid routinely includes microscopic evaluation of WBC and red blood cell count. The presence of crystals or bacteria is described as semi-quantitative grading. The WBC is particularly important as it gives a more objective measure of the degree of inflammation. However, WBC must be determined from a fresh synovial fluid sample, preferably within 2 hours of aspiration, which is not always possible. In the present study, the pulse-echo method was used. This technique does not require immediate use of the sample of synovial fluid. Our results showed that acoustic velocity was correlated with synovial WBC, viscosity, clarity, string test, and blood inflammation markers.

Acoustic velocity varies with the type of material it passes through and depends on the density and compressibility of the material. Substances with greater density and less compressibility will transmit sound at greater velocity. For instance, the acoustic velocity of air, fat, water, liver, blood, muscle, and cortical bone were 331, 1,450, 1,540, 1,549, 1,570, 1,585, and 4,080 m/s, respectively [10]. Alasaarela et al evaluated ultrasound propagation speed in arthritic synovial tissue and observed an increase in propagation speed from 1,515 to 1,565 m/s when the percentage of connective tissue increased from 30 to 90% [4]. The acoustic velocity also

### Table. Association of acoustic velocity of synovial fluid with various parameters

<table>
<thead>
<tr>
<th></th>
<th>No. of samples</th>
<th>Acoustic velocity* (m/s)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>WBC count</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 2,000</td>
<td>5</td>
<td>1,544.0 ± 1.5</td>
<td></td>
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<td>&gt; 2,000</td>
<td>21</td>
<td>1,550.0 ± 4.5</td>
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<td>1,545.9 ± 4.4</td>
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<td>&gt; 5 mg/dL</td>
<td>17</td>
<td>1,549.6 ± 4.4</td>
<td>0.118</td>
</tr>
<tr>
<td>Clarity</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Translucent</td>
<td>14</td>
<td>1,548.7 ± 4.6</td>
<td>0.001†</td>
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<tr>
<td>Opaque</td>
<td>7</td>
<td>1,549.8 ± 3.9</td>
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<td>String test</td>
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<tr>
<td>&gt; 5 cm</td>
<td>5</td>
<td>1,543.6 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>≤ 5 cm</td>
<td>2</td>
<td>1,548.2 ± 6.3</td>
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<td>1,545.0 ± 3.2</td>
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<td>1,550.6 ± 4.5</td>
<td>0.001**</td>
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<tr>
<td>Low</td>
<td>6</td>
<td>1,552.4 ± 3.5</td>
<td>0.012‖</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation. WBC = white blood cell count; CRP = C-reactive protein. †Translucent vs transparent; ‡opaque vs transparent; §string test > 5 cm vs ≤ 5 cm; ¶string test < 5 cm vs > 5 cm; ††variable vs high viscosity; ‖low vs high viscosity.

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increased as the temperature increased. These properties can be applied to assess the content of bio-fluids, such as synovial fluid, that can be obtained without invasive surgical procedures.

Our results showed that a higher acoustic velocity was associated with increased synovial WBC. The acoustic velocity was significantly different between samples with WBCs of less than 2,000/mm$^3$ and those with WBCs of more than 2,000/mm$^3$, which allowed differentiation between inflammatory and non-inflammatory synovial fluid. When the WBC data were compared in gout and non-gout groups, the association with increased WBC was stronger in the gout group ($r = 0.53$) than in non-gout groups. These results indicate that factors other than WBC, such as the amounts of crystals, red blood cells, and proteins or glycoproteins such as glycosaminoglycan, can influence the acoustic velocities of synovial fluids. Thus, synovial fluids obtained from homogenous patient subgroups should show better correlation on ultrasonic study.

In this study, the acoustic velocity was also correlated with the serum CRP. CRP is an acute-phase reactant and a non-specific inflammatory marker. CRP tends to appear sooner than an elevated ESR and to decrease more rapidly than ESR following therapy. Thus, it is a better indicator of acute inflammation than ESR. Our results indicate that acoustic velocity is more closely associated with serum CRP than with synovial WBC or blood ESR.

The acoustic velocity was higher in synovial fluid that was translucent or opaque, had a poor string test result, or had variable or low viscosity. These parameters are correlated with synovial fluid inflammation. The mean acoustic velocity of samples from the noninflammatory group was approximately 1,544 m/s, whereas in the inflammatory group, this was 1,550 m/s.

Ultrasonic measurements of synthetic crystals also showed increased acoustic velocity with increased concentration. The results of this study indicate that ultrasonic measurements of synovial fluid may be helpful in quantifying inflammation. The good correlations of acoustic velocities with synovial WBC, blood ESR, and serum CRP may be of value in the evaluation of cases where synovial fluid has become clotted or other inflammatory markers may not have been evaluated. This offers an alternative method to determine the extent of inflammation quantitatively and, hence, may help in the evaluation and management of arthritic conditions.

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**References**