SEROLOGICAL STUDIES ON MYOCARDIAL INFARCTION

BY

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Sometimes the physicians meet an atypical electrocardiogram in patients with old myocardial infarction and are confused about the decision to have or not the history of myocardial infarction1). Thus, in order to make up the weak point of electrocardiogram, the biochemical and serological methods are being introduced. Since C-reactive protein test has been introduced as the serological accessory diagnostic method of fresh myocardial infarction, there has been numerous reports made by Kroop and many others. However, in old myocardial infarction, any serological method has not been designed. In this regard the authors examined serologically the sera in patients with myocardial infarction and obtained the results that might be used clinically.

MATERIALS AND METHODS

Materials:

A total of 40 cases were studied. They were composed of 5 controls, 10 patients with angina pectoris, 2 with fresh myocardial infarction, 19 with old myocardial infarction and 4 with relapsed myocardial infarction. The diagnosis was confirmed by clinical history, clinical findings, laboratory results and electrocardiogram of standard 12 leads. Serum was sterilizedly prepared from 7-10 ml of whole blood in a clean glass centrifuge tube. Whole serological procedures were made under the sterilized conditions.

Methods:

1. C-reactive Protein Test. Bacto-C Protein Antiserum (Difco Laboratories, U.S.A.) was used for the determination that was of Anderson and McCarty2).

2. R.A. Test. The reagents, which were made of Hyland Lab., U.S.A., were used for the R.A. test. R.A. test was performed by the quick slide method that was designed by Singer and Plotz3,4).

3. Waaler-Rose Reaction modified by Heller5).

4. Boyden Test6) modified by Kumagai7). O-group human erythrocyte was used in stead of sheep's erythrocyte. Other technique was followed by

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the original method.

5. Precipitation Test (Ouchterlony Method\textsuperscript{10}). Antigen-Antibody reaction is two-dimensionally observed by this method. Here, that were composed from Coombs' serum and patient's serum as antibody, and O-group human liver infusion, its heart infusion, normal healthy human serum and human gamma-globulin as antigen.

6. Total Protein. It was determined by the refractometer made of Hitachi Co.\textsuperscript{9}.

7. Protein Fraction. The fraction of serum protein was estimated on paper-electrophoresis by amido-black method\textsuperscript{10}.

\textbf{RESULTS}

\textit{Part I.} Relationship of Myocardial Infarction with C-reactive Protein Test and Fraction II Test.

1. C-reactive Protein Test (Table 1).

C-reactive protein were positive in 2 patients with fresh myocardial infarction and 4 with relapsed myocardial infarction. It was negative in 19 patients with old myocardial infarction except one. Also, it was negative in all cases of angina pectoris.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
 & Case number & C.R.P. Test & R.A. Test \\
 & & $-$ & $+$ & $-$ & $+$ \\
\hline
Fresh myocardial infarction & 2 & 0 & 2 & 2 & 0 \\
Old myocardial infarction & 3 & 3 & 0 & 3 & 0 \\
\hspace{1cm} (non-transmural) & 16 & 15 & 1 & 2 & 14 \\
\hspace{1cm} (transmural) & 4 & 0 & 4 & 0 & 4 \\
Relapsed myocardial infarction & 4 & 0 & 4 & 0 & 4 \\
Angina pectoris & 10 & 10 & 0 & 10 & 0 \\
\hline
\end{tabular}
\caption{Relationship of Coronary Disease with C-reactive Protein Test and R.A. Test.}
\end{table}

2. Fraction II Test.

R.A. test and Waaler-Rose reaction modified by Heller were used as Fraction II test.

a. R.A. Test (Table 1).

In 3 patients with old non-transmural myocardial infarction, R.A. test were all negative. However, in 16 with old transmural myocardial infarction, 14, 87.6\%, were positive. On the other hand, all were negative in 10 with angina pectoris. In 4 with relapsed myocardial infarction all were positive for C-reactive protein test.

Thus, 3 cases that were positive in high degree on quick slide method
were selected and determined quantitatively by the dilution method (Table 2). Although agglutination was tiered up to 1:500 dilution in positive control serum of the reagent set and to 1:10 dilution in healthy negative control serum, all 3 cases were tiered over 1:20, especially 1:200 in one with old transmural myocardial infarction.

On the other hand, an agglutination between the latex without gammaglobulin and 1:20 diluted serum mentioned above as well as control positive serum have not occurred. So, the positive reaction on the R.A. test is not thought as a non-specific reaction for the latex.

b. Waaler-Rose Reaction modified by Heller (Table 3).

The method was performed in the above-mentioned 3 of myocardial infarction with positive R.A. test and in 3 of angina pectoris with negative R.A. test. The reaction was all positive in the myocardial infarction and observed to be equal degree to the agglutination in R.A. test. Also, that was negative in all 3 of angina pectoris.


R.A. test was performed about 1 to 40 months after the onset of myocardial infarction (Fig. 1).

In this connection, there is, as the example, one case with fresh transmural anterior myocardial infarction followed up. R.A. test became positive between 10 and 37 days after the onset (Fig. 2, 3a, b, c, d, e). Although C.R.P. test usually becomes negative in about two weeks after the onset, it became negative between 53 and 84 days after the onset and before the time of disappearance of the elevated STv, and negative Tv. Positive
Table 3. Waaler-Rose Reaction modified by Heller.

<table>
<thead>
<tr>
<th>Case</th>
<th>agglutination titer</th>
<th>Saline solution</th>
<th>R.A. Test agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A.</td>
<td>non-sensitized sheep erythro.</td>
<td>- - -</td>
<td>~</td>
</tr>
<tr>
<td>T.Y.</td>
<td>non-sensitized sheep erythro.</td>
<td>- - -</td>
<td>-</td>
</tr>
<tr>
<td>N.N.</td>
<td>sensitized sheep erythro.</td>
<td>+ + + + + + - - -</td>
<td>-</td>
</tr>
<tr>
<td>I.K.</td>
<td>non-sensitized sheep erythro.</td>
<td>- -</td>
<td>-</td>
</tr>
<tr>
<td>S.T.</td>
<td>sensitized sheep erythro.</td>
<td>- - - - - - -</td>
<td>-</td>
</tr>
<tr>
<td>Y.S.</td>
<td>non-sensitized sheep erythro.</td>
<td>- -</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. Differentiation in Coronary Disease.

<table>
<thead>
<tr>
<th></th>
<th>C.R.P. Test</th>
<th>R.A. Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh myocardial infarction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Old myocardial infarction</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1. Examination time from onset in positive group of R.A. test.
reaction of R.A. test was already observed at the time when there were recognized ascending ST and coronary T in electrocardiogram and positive reaction in C.R.P. test.

*Part II. Significance of R.A. Test in Myocardial Infarction.*

From Part I. of this examination results the authors supposed there was one to be called as “Reaction factor for Fraction II” in serum of old myocardial infarction.


Boyd test and Ouchterlony method were performed to study on whether “Reaction factor for Fraction II” was so-called auto-antibody for
Fig. 3. Serial Electrocardiograms in Case No. 30.
At this time, elevated ST\textsubscript{v}, depressed and negative T\textsubscript{v}, disappeared.

c. 111st day

Fig. 3. Serial Electrocardiograms in Case No. 33.
degenerative heart muscle or not.

Concerned with gamma-globulin as well as O-group human heart muscle infusion, Boyden test were negative in sera of all cases of myocardial infarction sera.

Coombs' serum was used for Ouchterlony method. Coombs' sera are the anti-human serum rabbit serum and anti-gamma-globulin serum.

![Diagram](image1)

**Fig. 4. Ouchterlony Method: with Coombs' serum.**

![Diagram](image2)

**Fig. 5. Ouchterlony Method: with old myocardial infarction patient's serum (positive reaction of R.A. test).**
Precipitation reaction lines were observed between Coombs' serum and O-group human heart muscle infusion, its liver infusion, gamma-globulin and healthy human serum (Fig. 4). However, precipitation reaction line was not observed between myocardial infarction patient's serum (positive R.A. test) and those above (Fig. 5).

From these results the existence of auto-antibody was doubtful.

2. Total Protein and Protein Fraction.

There was no significant difference between the negative group of R.A. test and the positive in the serum total protein (Fig. 6).

In the protein fraction, alpha-globulin and beta-globulin had no significant difference between the above two group. However, gamma-globulin was somewhat increased in the positive group (Fig. 7). It is suggested, therefore, there is dysproteinemia.

**DISCUSSION**

Since Kroop and Shackman demonstrated that serial C-protein determinations were of value in the subsidence of inflammatory processes and could be used to differentiate coronary insufficiency from myocardial infarction, it was reported that C-reactive protein constantly raised soon after myocardial infarction onset and disappeared in about two weeks after the onset. The authors observed (Table 1) that C-reactive protein raised in all two cases of fresh myocardial infarction patients and all 4 relapsed.
patients. On the other hand, all 10 cases in angina pectoris showed negative reactions of C-reactive protein.

As R.A. test was originally designed in order to examine serologically
the rheumatoid arthritis, the studies up-to-day mainly limited to these similar diseases. Our studies, however, have no relation with those studies. R.A. test were positive at the rate of 87.6% in old transmural myocardial infarction and negative in fresh myocardial infarction and angina pectoris (Table 1).

From the mention above, it concluded that Table 4 can be used to differentiate each other in coronary diseases. Further, the weak point of electrocardiogram will be considerably made up.

It is confirmed that the R.A. test mentioned above is in parallel with Waaler-Rose reaction and is not the non-specific reaction for the latex. Moreover, the dysproteinemia is supposed by the existence of hypergamma-globulinemia in sera of myocardial infarction patients with the positive R.A. test (Fig. 7). Generally it is thought that whether the change of serum protein is concerned with the increase and decrease of normal serum protein fraction or the development of paraproteins. In order to study the paraproteins, Boyd test, immuno-electrophoresis and Ouchterlony method are usually used.

Developed from these results, the authors have studied on whethere the "Reaction factor for Fraction II" was so-called auto-antibody for degenerative heart muscle affected by myocardial infarction or not. Coombs' serum was used for Ouchterlony method and precipitation reaction lines were observed between Coombs' serum and O-group human heart muscle infusion, its liver infusion, gamma-globulin and healthy human serum. However, the precipitation reaction lines were not observed between myocardial infarction patient's serum (positive R.A. test) and those above (Fig. 5). As Coombs' serum is anti-gamma-globulin, it is thought that there is no anti-gamma-globulin in myocardial infarction patient's serum. Also, Boyd test with gamma-globulin and O-group human heart muscle infusion were negative in all myocardial infarction sera. From these results the existence of auto-antibody was doubtful. Kleinsorge and Dornbusch\^{10} reported that 54.5% in myocardial infarction had auto-antibody from the results of positive Boyd test. However, their auto-antibody was not certified by Ouchterlony method and immuno-electrophoresis.

It seems that the cause of positive R.A. test in old myocardial infarction patient's sera is concerned with the macroglobulin as Singer's\^{17} advice.

**Summary**

1. C-reactive protein raised in all cases of fresh myocardial infarction.
2. R.A. test were positive at the rate of 87.6% in old transmural
myocardial infarction, and negative in fresh myocardial infarction and angina pectoris. This results are of value to differentiate the coronary disease each others.

3. Auto-antibody was not found in sera of myocardial infarction patients who were observed the positive reaction of R.A. test.

REFERENCE