Fibrinogen Concentration and Immunoelectrophoresis in the Internal Jugular Vein and the Corresponding Arterial Blood in Ischemic Brain Afflictions

BY OLDRICH J. KOLAR, M.D.,* AND MARK L. DYKEN, M.D.†

Abstract:
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In plasma specimens simultaneously obtained from the bulb of the internal jugular vein (IJV) and the brachial artery, fibrinogen, alpha-1 antitrypsin and alpha-2 macroglobulin concentrations were determined using the radial immunodiffusion technique. In 16 (22%) from the total 71 patients with various ischemic central nervous system afflictions studied, significant arteriovenous differences in the fibrinogen concentration were observed.

In 32 patients from this series, simultaneous internal jugular vein and arterial plasma fibrinogen immunoelectrophoresis was performed. In 16 (50%) of these patients, immunoelectrophoretic abnormalities in the internal jugular vein plasma fibrinogen precipitate were established. In 12 (37%) instances, the internal jugular vein immunoelectropherograms revealed abnormal precipitates. From these 12 patients, ten (83%) showed immunoelectrophoretic abnormalities in the fibrinogen precipitation arc and in seven (58%) instances significant arteriovenous differences in the fibrinogen concentration were established.

In the patients with abnormal precipitates in the internal jugular vein plasma immunoelectropherograms, the average interval of 13 days from the onset of neurological symptoms of the ischemic central nervous system affliction to the cannulation of the internal jugular vein was significantly shorter (P < 0.001) as compared to the average of 34 days in the patients with normal internal jugular vein immunoelectropherograms.

The examination of IJV plasma specimens by means of the techniques used appears to be prospectively important in two major areas: (1) as an auxiliary examination indicating localized thrombotic process in the vascular bed, primarily supplying the central nervous system; and on periodical examination, (2) as a laboratory indicator of resolution of the thrombosis.

Additional Key Words radial immunodiffusion technique alpha-1 antitrypsin alpha-2 macroglobulin ischemic central nervous system diseases

In the process of arterial mural thrombus formation, the platelet aggregation at the site of intimal injury is followed by deposition of fibrin in the area of the platelet mass. In the region of the developing thrombus, the proenzyme plasminogen is converted in plasmin by plasminogen activators. Plasmin solubilizes the fibrin and produces enzymatic degradation of fibrinogen with consequent formation of circulating fibrinogen-fibrin proteolysis complexes of 400,000 to one million molecular weight.

Experimental enzymatic degradation of fibrinogen results in production of two major and two to three minor fibrinogen constituents which were demonstrated by agar gel electrophoresis, column chromatography, ultracentrifugation, and the immunodiffusion technique. Plasmin degradation products of fibrinogen display electrophoretic mobility corresponding to the area extending from the alpha-1 to the gamma globulin region.
Exposure of fibrinogen to plasmin and other proteolytic or perhaps also hydrolytic enzymes\textsuperscript{11} produces progressive degradation of fibrinogen molecules which have been immuno-electrophoretically demonstrated using fibrinogen antiserum.\textsuperscript{12,13} Under normal conditions, the fibrinogen immuno-electropherogram shows one precipitation arc in the beta globulin region.\textsuperscript{8} Following approximately 30 minutes of plasmin degradation, formation of a second precipitation arc which extended into the gamma globulin region of the fibrinogen immuno-electropherogram was observed.\textsuperscript{18} By the end of the first hour of fibrinogen exposure to plasmin, an additional precipitation arc in the alpha\textsubscript{-}2 globulin area was immuno-electrophoretically demonstrated.

In human serum, several proteinase inhibitors have been identified. Thus far, two antiplasmins in the alpha-globulin plasma fraction have been recognized—the immediate inhibitor, alpha\textsubscript{-}2 macroglobulin, and the slowly reacting inhibitor, alpha\textsubscript{1}, antitrypsin, which produces irreversible plasmin inhibition.\textsuperscript{2,9} Plasmin and proteinase inhibitors form complexes which are eliminated from the circulation in less than 24 hours by reticuloendothelial cells in the liver, spleen and bone marrow. This might result in significant decrease in concentration of the serum alpha\textsubscript{-}2 macroglobulin. Under these conditions, the alpha\textsubscript{-}2 macroglobulin reaches normal serum level in several days or weeks.\textsuperscript{15}

Simultaneous qualitative and immunoelectrophoretic studies of fibrinogen in the internal jugular vein (IJV) and the corresponding arterial plasma in patients with ischemic central nervous system (CNS) diseases are not available. It is the purpose of this paper to present preliminary observations dealing with this topic.

**Methods**

Fifty-one males and 20 females with ischemic CNS afflictions were studied. The patients were hospitalized at the Cerebrovascular Research Unit of the Indiana University Medical Center, Indianapolis, Indiana, and neurologically examined by one or both authors. In all patients, the final diagnosis was established following four-vessel cerebral angiography, electroencephalography, brain scan, cerebral blood flow and biochemical laboratory studies.

Directional Doppler, Model 806, Parks Electronic Laboratory, Beaverton, Oregon, was used to determine the side of maximal linear venous flow. In 15 (21\%) of the patients studied, the left IJV was cannulated; in the remaining instances, the blood specimen was obtained from the bulb of the right IJV.

Six to 7 ml of blood from the bulb of one of the IJV and from one of the brachial arteries were obtained at the beginning of the cerebral blood flow measurements.\textsuperscript{16} The blood was collected in Vacutainer-Lavender tubes containing 10.5 mg of ethylene-diaminetetraacetic acid (K\textsubscript{3}) and 0.014 mg of potassium sorbate in purified water in a 15\% solution. The blood specimens were centrifuged ten minutes at 1,000 rpm and 2 ml of the supernatant were stored at $-20^\circ\text{C}$ until examined.

The concentration of fibrinogen, alpha\textsubscript{-}2 macroglobulin and alpha\textsubscript{1} antitrypsin in the IJV and the corresponding arterial plasma was examined by means of the single radial immunodiffusion technique.\textsuperscript{17} In preparation of the agar plates, antiserum produced by Hoechst Pharmaceutical Company, Kansas City, Missouri, Hyland, Division of Travenal Laboratory, Inc., Costa Mesa, California, and Behring Diagnostics, Woodbury, New York, was used. In all patients, at least two simultaneous examinations of the IJV and the corresponding arterial plasma specimens were performed and the arithmetic average of the measurements obtained was evaluated in the comparative studies. In the agar plates containing the anti-fibrinogen and the anti-alpha\textsubscript{-}2 antitrypsin antiserum, the diameter of the precipitates obtained was measured following 16 hours and in the plates containing the anti-alpha\textsubscript{-}2 macroglobulin antiserum after 24 hours of precipitation. The precipitation was performed at 5 to 6\(^\circ\text{C}\) in chambers with saline-soaked gauze sponges. The diameter of the precipitates was measured applying Finescale Comparator, Orange, California, and expressed in tenths of millimeter. The total protein concentration in the blood specimens was determined photometrically.

In 60 patients in this series, serum and cerebrospinal fluid (CSF) cellulo\textsubscript{-}e polvacetate electrophoresis and microimmuno-electrophoresis\textsuperscript{18} was performed using polyvalent rabbit antihuman serum and antisera to the immunoglobulin G (IgG), A (IgA) and to the Fab fragments of IgG.

In 32 patients from our series, the IJV and the corresponding arterial plasma were simultaneously studied immuno-electrophoretically applying a rabbit antiserum to human fibrinogen. In each patient, two to six fibrinogen immunoelectropherograms were performed.

**Results**

In 16 (22\%) of the patients examined, arteriovenous differences in the diameter of the plasma fibrinogen precipitate exceeding 9.9\% of the diameter of the larger precipitation circle were established. In four of these 16 cases, the arteriovenous difference in the diameter of the fibrinogen precipitate represented less than 15\%, in four it was less than 20\%, in four the disparity mounted to 20\% to 29\%, and four patients displayed differences in the measurements in the range over 29\%. In 10 (62\%) of the 16 instances, including three patients with greatest arteriovenous differences in the measurements obtained (63\%, 62\% and 60\%), the diameter of the fibrinogen precipitate on examination of the IJV plasma was smaller.

As compared to the remaining 59 cases studied, no significant differences in the clinical manifestations of acute ischemic CNS afflictions in the patients with arteriovenous disparity in the diameter
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of the fibrinogen precipitates were observed. Incidence of patients with neurological symptoms of a transient ischemic attack (TIA)\(^\text{10}\) was essentially the same in both groups.

In our series there was no increased incidence of arteriovenous disparities in the diameter of the plasma fibrinogen precipitate on cannulation of the IJV on the side corresponding to the hemispheral ischemic lesion. There was no correlation between the diameter of the fibrinogen precipitate and the total protein concentration in the IJV plasma specimens examined.

In our series, arteriovenous differences representing more than 9.9% in the diameter of the immunodiffusion precipitate of the alpha-1 antitrypsin and the alpha-2 macroglobulin were observed in only three and five patients, respectively. There was no correlation between the incidence of arteriovenous disparity in the concentration of fibrinogen and the antiplasmins studied.

Simultaneous immunoelectrophoretic examination of the IJV and the corresponding arterial plasma using fibrinogen antiserum revealed abnormalities in the venous fibrinogen immunoelectrophorograms in 16 (50%) of the total of 32 patients studied. Indication of formation of an additional arc of the fibrinogen precipitate in the alpha-2 globulin area (fig. 1, number 2) was found in two patients. Decreased density and/or shortening of the fibrinogen precipitate in the IJV plasma was established in 14 cases. In 12 patients, the IJV plasma immunoelectrophorograms revealed one to three or possibly four abnormal precipitates (AP) in the zone situated cathodally from the application well (fig. 1, numbers 2 to 13). In two instances, one precipitate corresponding to arcs numbers 3, 6 and 9 also was seen in the immunoelectrophorograms obtained from the corresponding arterial plasma specimens. In 10 of the 12 patients, the presence of the AP in the IJV plasma immunoelectrophrogram was associated with decreased density and/or with shortening of the fibrinogen precipitate (table 1).

In patients with AP in the IJV plasma immunoelectrophrogram, the average time interval of 13 days from the onset of neurological symptoms of the ischemic CNS affliction to the cannulation of the IJV was significantly shorter (P<0.001) as compared to the average of 34 days in the patients with normal IJV plasma immunoelectrophorograms (table 2). Two (17%) of the patients with AP in the IJV plasma immunoelectrophrogram showed transient neurological symptomatology with complete

<table>
<thead>
<tr>
<th>Day from the onset of neurological symptoms</th>
<th>Age</th>
<th>Sex</th>
<th>Number of precipitates</th>
<th>Abnormalities in the fibrinogen precipitates</th>
<th>Alpha-1 antitrypsin, %</th>
<th>Alpha-2 macroglobulin, %</th>
<th>Diagnosis</th>
<th>Artery</th>
<th>Bulb of the internal jugular vein</th>
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<tr>
<td>5</td>
<td>57</td>
<td>M</td>
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<td>RMC</td>
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<td>F</td>
<td>2</td>
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<td>1.5</td>
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<td>9</td>
<td>53</td>
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<td>D, SH</td>
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<td>48</td>
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<td>3</td>
<td>SH</td>
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<td>2.9</td>
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<tr>
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<td>49</td>
<td>M</td>
<td>3-4</td>
<td>E</td>
<td>1.4</td>
<td>9.4</td>
<td>0</td>
<td>RMC</td>
<td>LB</td>
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<tr>
<td>11</td>
<td>57</td>
<td>M</td>
<td>2</td>
<td>D</td>
<td>14.3</td>
<td>3.7</td>
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<tr>
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<td>76</td>
<td>M</td>
<td>2</td>
<td>D</td>
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<td>1.7</td>
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<td>RMC</td>
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<td>14</td>
<td>59</td>
<td>M</td>
<td>2</td>
<td>D</td>
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<tr>
<td>16</td>
<td>69</td>
<td>M</td>
<td>3</td>
<td>D</td>
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<td>3.0</td>
<td>5.0</td>
<td>LMC</td>
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\(\uparrow\) increased diameter of the precipitate on examination of the venous plasma
D — decreased density
SH — shortening of the precipitate
E — indication of doubling in the fibrinogen precipitate
ICA — internal carotid artery
RMC — right middle cerebral artery
LMC — left middle cerebral artery
TIA — transient ischemic attack
LIC — left internal carotid
RB — right brachial artery
LB — left brachial artery
RBIJ — right bulb of the internal jugular vein
LBIJ — left bulb of the internal jugular vein
VB — vertebral-basilar insufficiency
Plasma immunoelectropherograms from blood specimens obtained from the bulb of the internal jugular vein (V) and the brachial artery (A) using fibrinogen antiserum. -1- Normal findings. -2- Formation of an additional precipitation arc in the alpha globulin region (1) and presence of one precipitate (2) in the cathodal direction from the application well. A 58-year-old male with eight days' history of brain infarction. -3- Three precipitates (3, 4 and 5) in the cathodal direction from the application well and indication of increased density in the anodal segment of the fibrinogen precipitate. A 69-year-old male with 16 days' history of brain infarction. -4- Three precipitates of higher density (6, 7 and 8) in the cathodal direction from the application well. As compared to the arterial immunoelectropherogram (A) the fibrinogen precipitate in the internal jugular vein (V) immunoelectropherogram shows decreased density. A 64-year-old female with successive brain infarctions with the onset of neurological symptoms preceding the cannulation of the internal jugular vein by 34 days. -5- Three precipitates (9, 10 and 11) of higher density and almost identical position as compared to the previous two patients. A 48-year-old male with nine days' history of brain infarction. -6- Two faint precipitates in the cathodal direction from the application well (12 and 13) in presence of the fibrinogen precipitate of very low density. A 48-year-old male with nine days' history of brain infarction.
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TABLE 2

Quantitative Differences in the Fibrinogen, Alpha-2 Macroglobulin, and Alpha-1 Antitrypsin Concentrations and Immuno-electrophoretic Abnormalities in the Internal Jugular Vein and the Corresponding Arterial Blood Specimens

| Abnormal precipitates and immunoelectrophoretic fibrinogen abnormalities in the internal jugular vein plasma | Average difference in the diameter of the radial immunodiffusion precipitates obtained from the arterial and internal jugular vein blood in percentage |
|---|---|---|---|---|---|
| Number of patients | Average time interval in days from the onset of neurological symptomatology | Fibrinogen, % | Alpha-2 macroglobulin, % | Alpha-1 antitrypsin, % | Incidence of TIA, % |
| Abnormal precipitates and immunoelectrophoretic fibrinogen abnormalities in the internal jugular vein plasma | 12 | 13 | 20.6 | 3.7 | 1.4 | 16.6 |
| Immunoelectrophoretic fibrinogen abnormalities in the internal jugular vein plasma | 4 | 25 | 4.0 | 2.9 | 1.3 | 50.0 |
| Normal fibrinogen immunoelectrophorograms | 16 | 34 | 4.2 | 5.3 | 3.6 | 25.0 |

normalization of the clinical findings in less than 24 hours. In seven (58%) cases from the group with AP in the IJV plasma immunoelectropherograms arteriovenous differences in the diameter of the fibrinogen precipitates exceeded 9.9% of the larger one.

In the series of 32 patients in whom the IJV plasma was immunoelectrophoretically studied, arteriovenous disparity in the diameter of the fibrinogen was found in eight instances. In seven (87%) of these eight patients, AP and immunoelectrophoretic abnormalities in the fibrinogen precipitate in the IJV plasma immunoelectropherogram were observed.

In the patients studied, no significant correlation between the quantitative and/or immunoelectrophoretic changes in the plasma fibrinogen from the IJV and electrophoretic or immunoelectrophoretic serum and CSF abnormalities were established.

Discussion

Regarding our experience with the radial immunodiffusion technique based at the present time on quantitative serum IgG, IgA and IgM studies in more than 2,000 patients with various neurological disorders, the range of technical errors on repeated examination of identical serum specimens represents up to 10% in the diameter of the immunodiffusion precipitate.

In our studies, two examinations of the corresponding venous and arterial plasma specimens were performed in the same immunodiffusion plate and the arithmetic averages of the diameters of the immunodiffusion precipitates were compared. Arteriovenous difference in the diameter of the precipitates representing 10% or more was considered to be significant.

As compared to the corresponding brachial artery plasma specimens, differences in the fibrinogen concentration found in the IJV plasma in 22% of our patients with ischemic CNS afflictions suggest alteration in the fibrinogen metabolism, presumably originating in the neck or cerebral arteries and/or in the intracranial venous system. The significance of these findings remains unclear at the present time.

It was our impression that in a certain proportion of the patients studied, the quantitative and mainly qualitative differences in the arterial and IJV fibrinogen were beyond the sensitivity of the immunochemical method used.

In our series, the incidence of immunoelectrophoretic abnormalities in the IJV plasma fibrinogen was definitely higher (50%) compared to the occurrence of quantitative arteriovenous disparities in the fibrinogen concentration established in the IJV plasma (22%).

In 12 (37%) cases the IJV plasma immunoelectrophorograms showed AP. The occurrence of AP in ten patients (83%) was associated with decreased density and/or shortening of the fibrinogen precipitation arc, suggesting incorporation of a certain proportion of the circulating fibrinogen in the precipitates. In our material, the incidence of AP in the IJV plasma immunoelectropherograms was significantly higher in the first two weeks following the onset of neurological manifestations of an ischemic brain affliction.

There are practically unpredictable variations regarding the brain areas drained by the venous blood obtained on cannulation of the right or left bulb of the IJV. In our series, no increased incidence of arteriovenous differences in the fibrinogen concentration was established on examination of plasma specimens from the IJV homolateral to the ischemic hemispherical lesion.

Regarding the observation reported by Fletcher and Alkjaersig fibrinogen abnormalities found in the
IJV plasma of our patients with ischemic CNS afflictions were not completely unexpected. In our opinion, examination of the IJV plasma by means of the techniques used appears to be prospectively clinically important in two major areas: (1) as an auxiliary laboratory technique indicating a localized thrombotic process in the vascular bed primarily supplying the CNS, including the patients clinically classified as TIA; and on periodical examination (2) as a laboratory indicator of resolution of the in vivo thrombosis.

Using the techniques mentioned, we are in the process of examination of plasma specimens simultaneously obtained from the bulb of the IJV, brachial artery and antebrachial vein. So far, in three patients of this series, the AP associated with immunoelectrophoretic abnormalities in the fibrinogen precipitate were demonstrated only in the IJV plasma immunoelectropherograms. This observation would indicate that immunoelectrophoretic abnormalities in the IJV plasma immunoelectropherograms do not represent a manifestation of generalized blood hypercoagulability and might reflect changes in the fibrinogen metabolism associated with the thrombotic process in the CNS vascular bed.

Accessible techniques which would reveal quantitative and/or qualitative abnormalities in the circulating fibrinogen, reflecting the presence and possibly the phase of a thrombotic process occurring in the cerebrovascular circulation, represent a necessary prerequisite for therapeutic trials. The fibrinogen chromatographic method appears to be laborious and requires an unusual degree of technical expertise and skill.5 Relatively simple and well-established techniques used in our studies can be performed in every laboratory. However, further observations in well-documented patients with ischemic CNS afflictions are necessary to fully explore the clinical implications of our findings.

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References
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