Plasma Protein Fractions in Healthy Blood Donors Quantitated by an Automated Multicapillary Electrophoresis System

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Abstract

During the last decade, capillary electrophoresis (CE) has emerged as an important alternative to traditional analysis of serum and plasma proteins by agarose or celluloseacetate electrophoresis. CE analysis of plasma proteins can now be fully automated and also includes bar-code identification of samples, preseparation steps, and direct post-separation quantitation of individual peaks, which permits short assay times and high throughput. For laboratory work, it is important to have reference values from healthy individuals. Therefore, plasma samples from 156 healthy blood donors (79 females and 77 males) have been analyzed with the Capillarys instrument and the new high resolution buffer, which yields higher resolution than the β1–β2+ buffer. Albumin concentrations in samples are measured using nephelometry in order to assign protein concentrations to each peak. The 2.5 and 97.5 percentiles for both the percentages of different peaks and the protein concentrations in the peaks are calculated according to the recommendations of the International Federation of Clinical Chemistry on the statistical treatment of reference values. The Capillarys instrument is a reliable system for plasma protein analysis, combining advantages of full automation with high analytical performances and throughput.

Introduction

Approximately 200,000 to 250,000 serum or plasma protein electrophoresis are performed annually by Swedish hospital laboratories. The number is much higher in southern Europe. Analysis of plasma or serum proteins by agarose or capillary electrophoresis (CE) is routinely used to screen for M-components and to monitor the treatment of patients with M-components (1,2). Other important applications for protein electrophoresis are evaluation of immunological and inflammatory responses (acute phase reaction), following tissue injury, infarction, infection, or immune-related diseases (3,4). The two most widely used assays for the acute phase response in humans are C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). The ESR is influenced by several factors other than the inflammatory response, and CRP is sometimes considered to be too rapid and too sensitive for monitoring chronic diseases. Other markers of the acute phase response are α_1-antitrypsin, α_1-acid glycoprotein, haptoglobin, and fibrinogen. These proteins can be observed in a high-resolution CE electropherogram (5,6). Recently, CE systems have been adapted to allow the separation of plasma samples reducing preanalytical times. Assay time with the Capillarys CE system (Sebia, Paris, France) is less than 10 min. The new high resolution (HR) application for the Capillarys CE system yields an improved resolution in comparison with the previous buffer system (β1–β2+ reagent) and allows a good separation of plasma samples, thus making it possible to quantitate individual peaks from the electropherogram (6).

The aim of this study was to define the 2.5 and 97.5 percentiles reference interval of the different fractions in the electropherogram in a group of apparently healthy adult individuals. The albumin concentrations were measured by nephelometry and were then used to assign values in g/L to the individual peaks in the CE.

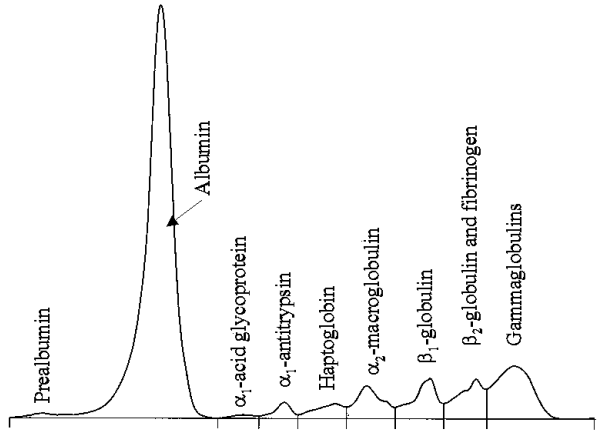

Figure 1. Electropherogram from a sample analyzed by Capillarys.

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Table I. Mean, Median, Lowest, and Highest Values and SD for the Study Population

Analyte	Mean	Median	Minimum	Maximum	SD
Whole population					
Albumin fraction, percentage	63.9	64.2	56.2	72.4	2.936
α_1-acid glycoprotein fraction, percentage	0.62	0.60	0.30	1.80	0.245
α_1-antitrypsin fraction, percentage	2.02	2.00	1.00	3.40	0.415
Haptoglobin fraction, percentage	3.07	3.05	1.40	5.60	0.832
α_2-macroglobulin fraction, percentage	4.16	4.20	2.60	6.10	0.696
β_1-globulin fraction, percentage	6.48	6.40	4.90	8.80	0.835
β_2-globulin fraction, percentage	4.72	4.70	3.00	6.70	0.693
Fibrinogen fraction, percentage	4.51	4.50	2.80	6.40	0.669
Gammaglobulin fraction, percentage	10.49	10.55	6.00	14.90	1.739
Albumin, g/L	41.4	41.8	30.0	50.0	3.521
α_1-acid glycoprotein fraction, g/L	0.40	0.37	0.16	1.16	0.163
α_1-antitrypsin fraction, g/L	1.31	1.29	0.68	2.15	0.275
Haptoglobin fraction, g/L	1.99	1.94	0.94	3.57	0.560
α_2-macroglobulin fraction, g/L	2.70	2.66	1.60	4.13	0.512
β_1-globulin fraction, g/L	4.19	4.17	2.79	5.73	0.596
β_2-globulin fraction, g/L	3.06	3.04	1.81	4.88	0.519
Fibrinogen fraction, g/L	2.93	2.91	1.56	4.51	0.528
Gammaglobulin fraction, g/L	6.82	6.76	3.72	10.18	1.371
Age	40.3	41.5	18.0	71.0	13.132
Females					
Albumin fraction, percentage	63.5	63.8	56.2	72.4	3.064
α_1-acid glycoprotein fraction, percentage	0.55	0.50	0.30	1.30	0.185
α_1-antitrypsin fraction, percentage	2.05	2.00	1.00	3.40	0.503
Haptoglobin fraction, percentage	3.15	3.10	1.40	5.60	0.902
α_2-macroglobulin fraction, percentage	4.42	4.40	2.80	6.10	0.676
β_1-globulin fraction, percentage	6.45	6.30	4.90	8.80	0.871
β_2-globulin fraction, percentage	4.71	4.70	3.00	6.20	0.683
Fibrinogen fraction, percentage	4.50	4.40	3.00	6.40	0.691
Gammaglobulin fraction, percentage	10.69	10.70	6.00	14.90	1.821
Albumin, g/L	41.0	41.0	32.0	48.0	3.208
α_1-acid glycoprotein fraction, g/L	0.35	0.33	0.16	0.88	0.125
α_1-antitrypsin fraction, g/L	1.32	1.28	0.68	2.15	0.330
Haptoglobin fraction, g/L	2.04	1.94	0.94	3.57	0.613
α_2-macroglobulin fraction, g/L	2.86	2.84	1.60	4.13	0.496
β_1-globulin fraction, g/L	4.17	4.15	3.01	5.71	0.610
β_2-globulin fraction, g/L	3.04	3.00	1.81	4.22	0.491
Fibrinogen fraction, g/L	2.92	2.88	1.81	4.51	0.523
Gammaglobulin fraction, g/L	6.92	6.89	3.77	10.05	1.342
Age	39.4	40.0	18.0	71.0	13.682
Males					
Albumin fraction, percentage	64.4	64.5	57.9	70.9	2.742
α_1-acid glycoprotein fraction, percentage	0.69	0.60	0.30	1.80	0.276
α_1-antitrypsin fraction, percentage	1.99	2.00	1.10	2.90	0.301
Haptoglobin fraction, percentage	2.98	2.90	1.60	4.70	0.752
α_2-macroglobulin fraction, percentage	3.90	3.80	2.60	5.30	0.615
β_1-globulin fraction, percentage	6.51	6.40	5.00	8.80	0.802
β_2-globulin fraction, percentage	4.74	4.70	3.50	6.70	0.708
Fibrinogen fraction, percentage	4.51	4.50	2.80	6.30	0.650
Gammaglobulin fraction, percentage	10.29	10.20	6.90	14.10	1.638
Albumin, g/L	41.8	42.6	30.0	50.0	3.793
α_1-acid glycoprotein fraction, g/L	0.45	0.41	0.18	1.16	0.183
α_1-antitrypsin fraction, g/L	1.29	1.30	0.77	1.97	0.205
Haptoglobin fraction, g/L	1.93	1.94	0.98	3.35	0.499
α_2-macroglobulin fraction, g/L	2.54	2.52	1.61	3.70	0.478
β_1-globulin fraction, g/L	4.22	4.21	2.79	5.73	0.583
β_2-globulin fraction, g/L	3.08	3.05	1.95	4.88	0.549
Fibrinogen fraction, g/L	2.94	2.91	1.56	4.45	0.537
Gammaglobulin fraction, g/L	6.72	6.58	3.72	10.18	1.401
Age	41.2	44.0	19.0	65.0	12.566

Experimental

Samples

Plasma samples were collected in gel tubes with lithium-heparin PST II (BD Vacutainer Systems, Plymouth, UK). The samples were obtained from 156 (79 females, 77 males) healthy adult blood donors at the University Hospital (Uppsala, Sweden). The study was approved by the local Ethical Board at Uppsala University (01-167).

Instrumentation

CE was performed using a Capillarys CE system using the new HR buffer. The instrument is equipped with eight capillaries, allowing a throughput of approximately 60 samples/h. The plasma samples are automatically diluted 1:5 with the migration buffer in dilution segments (40 µL sample to a final volume of 200 µL). Samples are then hydrodynamically injected for 4.0 s by anodic depression (injected volume ~ 1 nL). The separation is obtained by applying a voltage of 7 kV for 4 min in eight fused-silica capillaries (total/effective length 17.5/15.5 cm; inner diameter 25 µm). Measured throughput for the instrument was 60 samples/h when analyzing plasma samples.

The temperature is controlled by a Peltier element. The protein separation is performed at pH 9.9, and the protein fractions are detected by absorbance at 200 nm. Weekly cleaning of the capillaries by a washing solution is recommended by the manufacturer when analyzing serum samples and daily when analyzing plasma samples.

Analysis of albumin

Albumin was analyzed on a BN Prospec nephelometer (Dade Behring, Deerfield, IL) with reagents from the same manufacturer, including a calibrator traceable to CRM 470. The albumin assay had a total coefficient of variation of 1.7% at 38 g/L. The albumin value was used to assign values for individual peaks in the electropherogram.

Statistical calculations

Calculations of reference intervals were performed by bootstrap estima-

tion utilizing RefVal 4.0 (Department of Clinical Chemistry, Rikshospitalet, N-0027 Oslo, Norway). RefVal fulfils the recommendations of the International Federation of Clinical Chemistry on the statistical treatment of reference values (7–9).

Results

Description of the study population

Mean, median, lowest, and highest values, as well as standard deviations (SDs) for the studied fractions and age are presented in Table I.

Percentiles (2.5 and 97.5) of individual peaks in percentages of total absorbance

The 2.5 and 97.5 percentiles for the percentages of the different fractions were: albumin fraction, 58.1–69.9%; α_1-acid glycoprotein fraction, 0.3–1.3%; α_1-antitrypsin fraction, 1.2–3.0%; haptoglobin, 1.6–4.9%; α_2-macroglobulin fraction, 2.8–5.7 %; β_1-globulin fraction, 5.0–8.5%; β_2-globulin fraction, 3.6–6.2%; fibrinogen fraction, 3.2–5.9%; and gammaglobulin fraction, 7.4–13.9% for the entire group (Table II). All lower and upper limits for the female subgroup were within the 90% confidence intervals of the whole study group (Table III). For the males, only the upper limits for the α_1-antitrypsin fraction and α_2-macroglobulin were outside the 90% confidence intervals for the whole study group (Table IV).

Percentiles (2.5 and 97.5) of individual peaks (g/L)

The 2.5 and 97.5 percentiles for the percentages of the different fractions were: albumin fraction, 33.1–47.3 g/L; α_1-acid glycoprotein fraction, 0.18–0.86 g/L; α_1-antitrypsin fraction, 0.77–1.94 g/L; haptoglobin, 1.02–3.28 g/L; α_2-macroglobulin fraction, 1.73–3.80 g/L; β_1-globulin fraction, 3.12–5.50 g/L; β_2-globulin fraction, 2.14–4.17 g/L; fibrinogen fraction, 1.96–4.19 g/L; and gammaglobulin fraction, 4.19–9.73 g/L for the entire group (Table II). All lower and upper limits for both the female and male subgroups were within the 90% confidence intervals of the whole study group (Tables III and IV).

Discussion

The first automated multicapillary instrument designed for routine serum protein analysis by CE, the Paragon CZE2000

Table II. Calculated Upper and Lower Limits for the Reference Intervals and 90% Confidence Intervals* for all Blood Donors†

Analyte	Lower limit	Upper limit
Albumin fraction, percentage	58.1 (57.1–59.0)	69.9 (68.7–71.0)
α_1-acid glycoprotein fraction, percentage	0.3 (0.3–0.3)	1.3 (1.0–1.6)
α_1-antitrypsin fraction, percentage	1.2 (1.0–1.3)	3.0 (2.8–3.2)
Haptoglobin fraction, percentage	1.6 (1.5–1.7)	4.9 (4.5–5.4)
α_2-macroglobulin fraction, percentage	2.8 (2.6–3.1)	5.7 (5.2–6.2)
β_1-globulin fraction, percentage	5.0 (4.9–5.1)	8.5 (8.2–8.9)
β_2-globulin fraction, percentage	3.6 (3.3–3.8)	6.2 (6.1–6.4)
Fibrinogen fraction, percentage	3.2 (2.9–3.5)	5.9 (5.5–6.4)
Gammaglobulin fraction, percentage	7.4 (6.5–8.3)	13.9 (13.3–14.5)
Albumin, g/L	33.1 (31.5–34.6)	47.3 (46.6–48.0)
α_1-acid glycoprotein fraction, g/L	0.18 (0.17–0.20)	0.86 (0.67–1.05)
α_1-antitrypsin fraction, g/L	0.77 (0.72–0.82)	1.94 (1.83–2.06)
Haptoglobin fraction, g/L	1.02 (0.93–1.10)	3.28 (3.06–3.51)
α_2-macroglobulin fraction, g/L	1.73 (1.59–1.89)	3.80 (3.54–4.05)
β_1-globulin fraction, g/L	3.12 (2.97–3.26)	5.50 (5.28–5.71)
β_2-globulin fraction, g/L	2.14 (1.97–2.32)	4.17 (3.94–4.40)
Fibrinogen fraction, g/L	1.96 (1.81–2.10)	4.21 (3.84–4.57)
Gammaglobulin fraction, g/L	4.19 (3.63–4.75)	9.73 (9.13–10.33)

* In parentheses.
† n = 156.

Table III. Calculated Upper and Lower Limits for the Reference Intervals and 90% Confidence Intervals* for Female Blood Donors†

Analyte	Lower limit	Upper limit
Albumin fraction, percentage	57.5 (56.1–58.8)	70.5 (68.3–72.7)
α_1-acid glycoprotein fraction, percentage	0.3 (0.3–0.3)	1.1 (0.8–1.4)
α_1-antitrypsin fraction, percentage	1.1 (1.0–1.2)	3.2 (2.9–3.4)
Haptoglobin fraction, percentage	1.5 (1.3–1.7)	5.3 (4.8–5.8)
α_2-macroglobulin fraction, percentage	3.1 (2.8–3.5)	6.0 (5.6–6.3)
β_1-globulin fraction, percentage	5.0 (4.8–5.2)	8.5 (8.1–9.0)
β_2-globulin fraction, percentage	3.4 (2.9–3.8)	6.1 (5.9–6.3)
Fibrinogen fraction, percentage	3.2 (2.9–3.4)	6.0 (5.5–6.6)
Gammaglobulin fraction, percentage	6.9 (5.7–8.2)	14.3 (13.4–15.2)
Albumin, g/L	33.6 (31.4–35.7)	47.2 (46.2–48.3)
α_1-acid glycoprotein fraction, g/L	0.17 (0.15–0.19)	0.73 (0.57–0.90)
α_1-antitrypsin fraction, g/L	0.73 (0.67–0.80)	2.03 (1.86–2.20)
Haptoglobin fraction, g/L	0.99 (0.88–1.10)	3.42 (3.19–3.66)
α_2-macroglobulin fraction, g/L	1.88 (1.56–2.20)	3.95 (3.68–4.23)
β_1-globulin fraction, g/L	3.10 (2.95–3.25)	5.45 (5.16–5.74)
β_2-globulin fraction, g/L	2.11 (1.80–2.41)	4.01 (3.80–4.23)
Fibrinogen fraction, g/L	1.97 (1.79–2.14)	4.07 (3.65–4.49)
Gammaglobulin fraction, g/L	4.23 (3.67–4.79)	9.53 (8.73–10.33)

* In parentheses.
† n = 79.

Table IV. Calculated Upper and Lower Limits for the Reference Intervals and 90% Confidence Intervals* for Male Blood Donors†

Analyte	Lower limit	Upper limit
Albumin fraction, percentage	59.0 (57.7–60.3)	69.7 (68.5–70.9)
α_1-acid glycoprotein fraction, percentage	0.3 (0.3–0.3)	1.5 (1.1–1.9)
α_1-antitrypsin fraction, percentage	1.3 (1.0–1.5)	2.7 (2.5–3.0)
Haptoglobin fraction, percentage	1.6 (1.5–1.7)	4.5 (4.2–4.8)
α_2-macroglobulin fraction, percentage	2.7 (2.5–2.9)	5.1 (4.9–5.4)
β_1-globulin fraction, percentage	5.1 (4.8–5.3)	8.5 (8.1–8.9)
β_2-globulin fraction, percentage	3.7 (3.5–3.8)	6.4 (6.0–6.7)
Fibrinogen fraction, percentage	3.2 (2.8–3.7)	6.0 (5.5–6.5)
Gammaglobulin fraction, percentage	7.6 (6.9–8.2)	13.5 (12.7–14.3)
Albumin, g/L	32.2 (29.5–35.0)	48.0 (45.9–50.1)
α_1-acid glycoprotein fraction, g/L	0.19 (0.18–0.21)	1.00 (0.76–1.25)
α_1-antitrypsin fraction, g/L	0.82 (0.69–0.96)	1.83 (1.66–1.99)
Haptoglobin fraction, g/L	1.06 (0.97–1.15)	3.07 (2.70–3.44)
α_1-macroglobulin fraction, g/L	1.69 (1.57–1.81)	3.56 (3.38–3.74)
β_1-globulin fraction, g/L	3.06 (2.75–3.36)	5.54 (5.27–5.82)
β_2-globulin fraction, g/L	2.13 (1.92–2.34)	4.37 (3.89–4.86)
Fibrinogen fraction, g/L	1.85 (1.52–2.18)	4.25 (3.80–4.70)
Gammaglobulin fraction, g/L	4.14 (3.42–4.86)	9.87 (9.24–10.49)

* In parentheses.
† $n = 77$.

(Beckman Instruments, Brea, CA), was commercialized in 1994 (10). The Capillarys is the second generation of automated multicapillary instruments for serum or plasma protein analysis. It is fully automated with bar-code identification for patients and racks, preseparation steps, and direct postseparation quantitation of individual peaks. CE provides simultaneous information on several protein fractions and can be used for evaluation of immunological and inflammatory responses and for the detection of M-components. The Capillarys CE system allows a good separation of α_1-antitrypsin, α_1-acid glycoprotein, and haptoglobin in serum and plasma samples, thus making it possible to quantitate these proteins from the electropherogram (6). There are several factors, other than the acute phase response, that can influence individual plasma proteins. Thus, it may advantageous to determine several acute phase proteins as in CE. Low α_1-antitrypsin may be attributable to genetic deficiency and increased levels may be caused by liver damage or oestrogen therapy (11). α_1-Acid glycoprotein (12) is influenced by the glomerular filtration rate with increased levels in plasma from patients with kidney damage, and haptoglobin is low in plasma from patients with liver cirrhosis or haemolysis (13–15). To be able to interpret the individual peaks in the electropherogram, it is essential to have knowledge of the variation of individual peaks in healthy individuals. We have, thus, studied the variation of individual protein peaks in healthy blood donors.

The health care strives to shorten patient turnaround times (TAT) and thus also laboratory test TAT. The transit from celluloseacetate and agarose electrophoreses to CE has made it possible to significantly shorten TAT. The use of lithium-heparin PST tubes eliminates delay because of sample clotting. With PST tubes, preanalytical automation, CE, computerized interpretation, and an electronic request–result system, it should be possible to provide test results within 30 min from the time that the sample arrives to the laboratory. This is superior to ESR and similar to CRP TAT. In the future, we believe that plasma protein electrophoresis with capillaries will be an interesting alternative to ESR, providing shorter TATs and more information to the clinician.

Conclusion

Reported here are the 2.5 and 97.5 percentiles for individual peaks in the electropherogram for healthy blood donors. The calculations were performed according to the recommendations of the International Federation of Clinical Chemistry (Milano, Italy) on the statistical treatment of reference values.

Acknowledgments

We greatly appreciate the technical assistance of Ms. Charina Brännström.

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Manuscript received February 16, 2005.