Gc-globulin in liver disease

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BACKGROUND GC-GLOBULIN

The α-2-globulin Gc-globulin (other names: group-specific component, vitamin D-binding protein) is a multifunctional protein [8-10], and its main physiological importance is probably binding of actin. Gc-globulin scavenges monomeric actin and together with gelsolin constitutes the extracellular actin scavenger system [11].

Gc-globulin was first described in 1959 [12]. It is encoded for on the long arm of chromosome 4 (4q11-13). The Gc-globulin gene has been identified [13]; the protein consists of 458 amino acid residues (and a 16 amino acid tail) and contains 14 disulfide bridges [9,14]. The molecular weight is approximately 56,000 Daltons, but the exact weight depends on the amount of glycosylation.

The serum concentration of Gc-globulin in normal individuals is approximately 300 to 500 mg/L (5.4 to 8.9 \times 10^{-6} M), thus constituting the extrahepatic pool of vitamin D metabolites whereas domain III contains the actin-binding site with a very good structural fit to a large groove between actin subdomain 1 and 3 [32]. The intermolecular contact between the 2 proteins is large (3400-3600 Å²) [32,33].

Gc-globulin kinetics has not been studied in great detail. Gc-globulin is synthesized almost solely in the liver [8], although minimal Gc-globulin mRNA expression (in the rat) has also been documented in other tissues, including the kidney, yolk sac, and testis suggesting a minimal extrahepatic synthesis [26]. In healthy human volunteers the exchangeable pool of Gc-globulin was evaluated to be 3 gram [34]. The half-life of uncomplexed Gc-globulin is approximately 48 hours in man [34], 17 hours in the rabbit [35], and 10 hours in the rat [36]. Actin-complexed Gc-globulin has a much shorter half-life, ~ 60 minutes in the rabbit [35] and 30 minutes in the rat [36].

THE EXTRACELLULAR ACTIN SCAVENGER SYSTEM

Disruption of the integrity of the cell membrane will cause release of actin to the extracellular space. This disruption may be part of normal cell turnover or may stem from cellular necrosis caused by pathologic conditions leading to cell necrosis [11]. Release of actin from damaged or necrotic cells into the circulation may have severe side effects for the organism [49]. In a very important study, Haddad et al demonstrated that infusion of high doses of G-actin in the rabbit caused rapid and fatal formation of massive actin filament-containing thrombi in arterioles and capillaries of pulmonary veins and there was also evidence of endothelial injury [49]. Thrombi formation was not observed when the same amount of G-actin was preincubated with Gc-globulin. Further evidence for the deleterious effects of actin was demonstrated by Erukhimov et al showing that actin from necrotic cells could produce a direct injury to pulmonary endothelial cells [50].

These and other observations lead to the suggestion of the presence in plasma of an extracellular actin scavenger system, consisting of two plasma proteins: Gc-globulin and gelsolin [11;35;51;52]. Gelsolin, synthesized in skeletal muscle [53], depolymerizes polymeric actin, F-actin, by capping [54], annealing, and severing [42,55] the protein at a 1:2 molar ratio [56], whereas Gc-globulin binds with high affinity (Kd= 10^{-9} M) to monomeric actin at a 1:1 molar ratio, thus forming a Gc-globulin:actin complex [57]. The complex is cleared by parenchymal and endothelial cells [58] or Kupffer cells in the liver [59] (Figure 1).

OTHER FUNCTIONS OF GC-GLOBULIN

Gc-globulin is a multifunctional protein [31]. Its presumed physiologic functions are listed in Table 1.

Vitamin D-binding: Binding of vitamin D metabolites (primarily 25-OH vitamin D and 1,25-di-OH vitamin D) was the first described function of Gc-globulin, hence, the synonym vitamin D-binding protein (VDBP or DBP) [60,61]. Gc-globulin’s sterol-binding (vitamin D-binding) site is located at the amino-terminal end of the protein (domain I), as opposed to the actin-binding site at the
carboxy-terminal end (domain III) [62]. Binding of sterols does not affect the actin-binding property or capacity [62]. Vitamin D-binding occupies less than 5% of the normal sterol-binding capacity [8], and Gc-globulin levels do not correlate with levels of vitamin D metabolites [63].

Precursor for macrophage activating factor: In 1991, Yamamoto et al. described for the first time the role of Gc-globulin as a precursor of macrophage activating factor (Gc-MAF). In a series of experiments [64-66], this group described the conversion of Gc-globulin to MAF; Gc-globulin is modified by the combined action of membrane-bound β-galactosidase of B-lymphocytes and sialidase of T-lymphocytes to form Gc-MAF [64]. Gc-MAF acts as a switch to turn on macrophage activity at sites of infection and inflammation [67] and may cause apoptosis of these macrophages when they are no longer needed [68]. Some AIDS and cancer patients produce α-N-acetylgalactosaminidase, an enzyme that deglycosylates Gc-globulin, inhibiting the formation of Gc-MAF [69-71], and possibly contributing to the immunosuppression observed in these patients.

Also, Gc-MAF has direct antiangiogenic effects on endothelial cells [72;73] and an overexpression of Gc-globulin has been observed in tumor-bearing breasts [74].

Co-chemotactic effect for C5a and C5a des Arg: Gc-globulin enhances the neutrophil chemotactic effect of C5a and C5a des Arg for neutrophils and macrophages [75-78]. The mechanism seems to be regulated by elastase from neutrophils [79] and is related to a direct binding to C5a des Arg, since the Gc-globulin:C5a des Arg complex increases the number of C5a des Arg molecules/unit on the polymorphonuclear leucocytes [80]. Gc-globulin's C5a chemotactic cofactor function is mediated by CD44 and annexin A2, both involved in cell movement [81;82].

Natural killer cell enhancement: Anti-Gc-globulin antiserum inhibits the activity of natural killer (NK) cells on peripheral blood lymphocytes in vitro [83;84]. This inhibitory effect is blocked by addition of purified Gc-globulin. Thus, Gc-globulin seems to be associated with NK cytolysis in the post-binding cytolytic phase.

Binding of arachidonic acid and endotoxin: Under normal circumstances, 75-80% of serum arachidonic acid, parent molecule for the cyclooxygenase pathway, is bound to Gc-globulin [85-87]. Addition of Gc-globulin to protein preparations leads to a 40% decreased endotoxin neutralizing activity [88].

Others: Surface-bound Gc-globulin has been observed in a number of cells, including monocytes [89], B-lymphocytes [90;91], human placental trophoblasts, and neutrophils [92]. The Gc-globulin is probably plasma-derived [93], and the physiologic importance may be related to cell differentiation [94]; however, this issue is not yet clarified. Other functions include the recent finding, that Gc-MAF can stimulate osteoclast activity and bone resorption in an extracellular calcium-dependent way [95].

In conclusion, Gc-globulin is a multifunctional protein, mainly associated with the nonspecific innate immune system, vitamin D-binding, and actin-scavenging. The priority of these functions is not yet clear. Further, to what extent each function influences the other functions remains to be studied.

**ANALYSIS OF GC-GLOBULIN**

The following Gc-globulin definitions are useful when reading this thesis:

- **Total Gc-globulin**: the total concentration of Gc-globulin in serum.
- **Complex ratio**: the percentage of total Gc-globulin complexed to actin.
- **Free Gc-globulin**: the concentration of Gc-globulin not complexed to actin. Can be calculated as total Gc-globulin x (1 – complex ratio).
- **Bound Gc-globulin**: the concentration of Gc-globulin complexed to actin. Can be calculated as total Gc-globulin x complex ratio.

Several methods have been developed to measure serum Gc-globulin concentrations. In normal individuals, the mean and range for
ACETAMINOPHEN (PARACETAMOL) OVERDOSE

Acetaminophen is a very safe drug when ingestion does not exceed the daily recommended maximum of 4 gram/day. However, acetaminophen is also a dose-dependent hepatotoxin and its therapeutic index is very low. The typical pathological finding is centrilobular (zone III) necrosis where up to 90 per cent of hepatocytes may be necrotic [114-116]. Aminotransferase levels are usually very high [114]. Hepatotoxicity is more likely to occur if the antidote N-acetylcysteine (NAC) is instituted late after ingestion or if the patient is a chronic alcoholic [117;118].

Development of hepatic encephalopathy (and therefore, by definition, acute liver failure (ALF)) is also related to delay to NAC treatment [119] and is most likely to occur in patients with accidental overdose [120]. Acetaminophen overdose is the commonest cause of ALF in Denmark [121], the United Kingdom [122], and now also in the United States [123].

Experimental studies on acetaminophen-induced ALF have shown the actin-scavenger system to be stressed, as evaluated by decreased Gc-globulin levels. Also, a very high proportion of Gc-globulin in actinsterilized complexes (i.e., a high complex ratio) was observed in animals with severe liver damage [98;99].

So far, 5 clinical studies on Gc-globulin in acetaminophen overdose have been published (Table 3). Patients with ALF have been in-

### Table 2. Methods for determining serum Gc-globulin and complex ratio.

<table>
<thead>
<tr>
<th>Method</th>
<th>Ref.</th>
<th>Gc-globulin (mg/L)</th>
<th>Complex ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single radial immunodiffusion</td>
<td>(97)</td>
<td>422 (315-523)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(101)</td>
<td>522 ± 62 (460-584)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(63)</td>
<td>340 ± 61</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(102)</td>
<td>294 ± 3</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>292 ± 33</td>
<td>N/A</td>
</tr>
<tr>
<td>Rocket immunoelectrophoresis</td>
<td>(96)</td>
<td>357 ± 132 (273-529)</td>
<td>32 ± 8 (20-44)</td>
</tr>
<tr>
<td></td>
<td>(103)</td>
<td>393 ± 65</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(104)</td>
<td>404 ± 124</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>342 ± 61 (240-482)</td>
<td>13 ± 13 (0-27)</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>365 ± 57 (265-390)</td>
<td>11 ± 12 (2-28)</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>340 ± 35 (265-390)</td>
<td>13 ± 8 (2-28)</td>
</tr>
<tr>
<td>Radioimmunoassay</td>
<td>(105)</td>
<td>347 ± 5</td>
<td>N/A</td>
</tr>
<tr>
<td>Western blot</td>
<td>(106)</td>
<td>288 (138-427)</td>
<td>N/A</td>
</tr>
<tr>
<td>Nephelometry</td>
<td>(106)</td>
<td>292 (163-509)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(107)</td>
<td>394 (320-460)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>350 (500)</td>
<td>N/A</td>
</tr>
<tr>
<td>ELISA</td>
<td>(108)</td>
<td>355 ± 99 (220-606)</td>
<td>N/A</td>
</tr>
<tr>
<td>Turbidimetry</td>
<td>(109)</td>
<td>305 (176-623)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A = not available.

### Table 3. Gc-globulin in acetaminophen overdose, clinical studies.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Analysis method</th>
<th>Observation period</th>
<th>Characteristics and findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al (97)</td>
<td>47*</td>
<td>PAGE</td>
<td>Daily samples, unclear period</td>
<td>All had HE. Admission Gc was 55 ± 13 mg/L in nonsurvivors and 82 ± 11 mg/L in survivors. Complex ratio was measured but not reported. Free Gc calculated</td>
</tr>
<tr>
<td>Schiedt et al (1) Copenhagen</td>
<td>18</td>
<td>RIEP</td>
<td>Every 3 hours, up to 30 hours after admission</td>
<td>No HE (n=10)</td>
</tr>
<tr>
<td>Schiedt et al (2) Copenhagen</td>
<td>18</td>
<td>RIEP</td>
<td>Admission</td>
<td>All had HE. Gc: 174 ± 91 mg/L Complex ratio: 46 ± 31%</td>
</tr>
<tr>
<td>Schiedt et al (6) Copenhagen</td>
<td>84</td>
<td>RIEP</td>
<td>Twice daily, entire hospital stay</td>
<td>Non-HEPTOX (n=32)</td>
</tr>
<tr>
<td>Schiedt et al (7) U.S.A. (multi-center)</td>
<td>76</td>
<td>Nephelometry</td>
<td>Day 1 and 2</td>
<td>All had HE. Gc: 114 (range 34-307) mg/L</td>
</tr>
<tr>
<td>Schiedt et al (3) Copenhagen</td>
<td>18</td>
<td>RIEP</td>
<td>Admission</td>
<td>All had HE. Gc: 174 ± 91 mg/L Complex ratio: 46 ± 31%</td>
</tr>
</tbody>
</table>

HE = hepatic encephalopathy. HEPTOX = ALT over 1,000 U/L but no HE. Gc = total Gc-globulin. Non-HEPTOX = ALT below 1,000 U/L and no HE. PAGE = polyacrylamide electrophoresis. RIEP = rocket immunoelectrophoresis. *) 39 of these patients had acetaminophen-induced ALF.
cluded in all these studies. Only 2 studies have reported Gc-globulin levels in patients without ALF [1,6]. These 2 studies have also described the temporal profile of serum Gc-globulin in acetaminophen overdose. In study [1], patients were followed for approximately 24 hours after inclusion in the study. Levels were lower among patients with hepatic encephalopathy than in those without. Gc-globulin levels did not change much over that period of time (Figure 2). In study [6], patients were followed over the entire hospital stay and several important observations could be made. Patients were divided into 3 groups according to degree of liver injury: 1) no or minimal injury, 2) moderate injury with high aminotransferase levels but no ALF, and finally 3) patients with ALF. Total and free Gc-globulin concentrations and complex ratio levels were unaffected in group 1 (Figure 3 and Figure 4), in contrast to the decrease in plasma coagulation factor II, VII, X activities (Figure 5) probably mediated by the anti-coagulant effect of the antidote NAC [124,125]. In contrast, patients in group 2 displayed signs of affection of the actin scavenger system, since total and free Gc-globulin levels fell to less than half of normal values, with nadir values occurring ca. 3 days after acetaminophen ingestion at the same time where complex ratio and aminotransferase levels peaked (Figures 3-5). These changes were more accentuated in group 3 where nadir and peak levels were even more abnormal. For groups 2 and 3 levels gradually normalized hereafter even though they were not yet in the normal range 7 days into overdose.

In general, total Gc-globulin levels are decreased in all patients with hepatotoxicity (Table 3). Patients with ALF have total Gc-globulin concentrations of approximately 100 mg/L which is less than one third of normal values.

Bound Gc-globulin remained normal at all times for all 3 groups. If one excludes the possibility of a methodology bias (the fact that bound Gc-globulin is not measured directly but is a product of 2 measured variables) then this could indicate that bound Gc-globulin concentration is narrowly regulated. I speculate that bound Gc-globulin levels may regulate Gc-globulin:actin complex metabolism even in the failing liver. However, the exact mechanism of the uptake of Gc-globulin:actin complexes in the liver is largely unknown so further studies should elucidate this.

To summarize, levels of Gc-globulin and actin complex ratio are affected in patients with acetaminophen overdose if hepatotoxicity is present, and patients with ALF have the most depressed serum levels. The time profile shows a close correlation between peak complex ratio and peak aminotransferase levels indicating that actin released from the necrotic hepatocytes contribute to the stress on the actin scavenger system in acetaminophen overdose.

**ACUTE LIVER FAILURE (FULMINANT HEPATIC FAILURE) AND PROGNOSIS**

It is safe to say that acute liver failure (ALF) is one of the most dramatic conditions in medicine. The failing liver leads by definition to hepatic encephalopathy within a short time after initial symptoms [126] and may also lead to a cascade of organ failures including renal failure, circulatory collapse, and pulmonary dysfunction. Further, severe infections, deep coagulopathy, and the risk of cerebral edema, intracranial hypertension, and cerebral herniation adds to the picture of an extreme disease entity. Not surprisingly, the mortality rate in ALF has historically been very high, with survival being the exception to the rule [127-130]. The increased use of intensive...
care monitoring and therapy and the advent of liver transplantation as a treatment option has improved prognosis considerably; however, mortality rates still remain at approximately 40 to 50%, even in the most experienced centers [123;131;132]. Favorable etiologies include acetaminophen, hepatitis A, pregnancy, and shock liver where the spontaneous survival rate (i.e., survival without liver transplantation) is greater than 50% as compared to a lower than 25% spontaneous survival rate for all other etiologies [123;133;134].

Serum Gc-globulin in ALF was first studied by Lee & Galbraith's group. Their initial reports, including relatively few patients, documented dramatic changes in Gc-globulin levels and complex ratio values [96;101;109], suggesting a severe reduction of Gc-globulin's actin-scavenging capacity. These studies were supported by similar findings in animal models of acetaminophen-induced ALF where, typically, total Gc-globulin levels were low and actin complex ratios were high and the time of the extremes correlated with the peak of aminotransferase levels [98;99].

So far, eleven clinical studies regarding Gc-globulin and ALF have been published (Table 4). Eight studies [1;2;6;7;96;101;106;109] have reported on total Gc-globulin levels. The results were very similar among the studies; Gc-globulin concentrations were reduced to between 25% and 49% of normal (Table 4). Free Gc-globulin levels (reported in 5 studies) were even lower, between 12% and 26% of normal, and complex ratios (reported in 5 studies) were elevated in all papers. Thus, the stress on the actin scavenger system in ALF seems very obvious.

Since the hepatic necrosis is so overwhelming in acetaminophen hepatotoxicity one would a priori assume Gc-globulin levels in this group to be lower than in the nonacetaminophen group. In fact, the opposite is true, since patients with acetaminophen-induced ALF have higher Gc-globulin concentrations than those patients with nonacetaminophen-induced ALF [2;7]. This could be due to a better prognosis in acetaminophen-induced ALF where the spontaneous survival chances are greater than 50% compared to a lower than 25% chance in nonacetaminophen-induced ALF [123;134]. In fact, spontaneous survivors of acetaminophen etiology had the same Gc-globulin levels as survivors of nonacetaminophen etiology in one study [7] – whereas there was a significant difference in Gc-globulin levels among nonsurvivors of the 2 groups. Another explanation for the increased Gc-globulin levels in acetaminophen-induced ALF may be that this disease is a »single-hit« disease where acetaminophen – or rather its highly reactive metabolite NAPQI – causes severe hepatocellular damage [135;136]. However, further damage is stopped once antidote treatment is instituted, in contrast to the continuous damage inflicted to the liver by for instance acute viral hepatitis B or by acute Wilson's disease.

Several prognostic models are available to determine outcome in ALF, including the British King's College Hospital criteria [137], the French Clichy criteria [138], plasma coagulation factors [139], arterial lactate [140], arterial ammonia [141], phosphate [142], and serum levels of alpha-fetoprotein [143;144]. The King's College Hospital criteria are still the most commonly used, despite being almost 20 years old [137]. However, the predictive accuracy of a model seems to decrease when applied on patients from other regions or countries than where they originated [145-147].

Three studies have reported on Gc-globulin and prognosis in ALF (Table 5), and the final results of an ongoing study from the USA are pending [148]. Preceding these studies, one paper reported the
value of actin complex ratio in seven patients with ALF [101], and found that nonsurvivors had higher complex ratio than survivors. Two studies [2,7] described the prognostic value of total Gc-globulin concentrations. In general, the prognostic value was better for nonacetaminophen patients. The prognostic cutoff levels for total Gc-globulin were quite similar in the 2 studies, 100 mg/L and 80 mg/L, respectively. In fact, in one study [7], there was no difference in total Gc-globulin levels between survivors and nonsurvivors of acetaminophen-induced ALF. For the nonacetaminophen groups, the positive prognostic values were 79% and 85%, respectively, whereas the negative predictive values were lower, 60% and 43%, respectively (Table 5). One study [97] reported on the prognostic value of free Gc-globulin and here the test seems to yield prognostic information in acetaminophen patients also. The prognostic cutoff level for free Gc-globulin was 34 mg/L and day 2 data seemed to give better prognostic information than admission levels. In the above mentioned abstract [148] the prognostic cutoff level was 40 mg/L and thus very close to that reported in Lee et al’s study. Even though the predictive accuracy of Gc-globulin was rather low, it was in the same range as that of the King’s College Hospital criteria in all 3 studies, demonstrating the imprecision of all prognostic markers.

The ideal prognostic marker is 100% accurate, with a perfect discrimination between positives and negatives. Unfortunately, no such marker exists. With the advent of acute liver transplantation as a treatment option it is even more important to have accurate prognostic markers, since we don’t want to transplant those patients who would survive spontaneously. Conversely, we want to make an early request for a liver donor in patients with a low likelihood of survival. Most studies who described prognosis in ALF give data on sensitivity and specificity. A meta-analysis showed the sensitivity to be lower than the specificity in most studies [149] meaning that it is apparently easier to identify survivors than nonsurvivors. For the clinician, however, these data are less useful since they are not transplanted patients. Of course, these patients could be excluded from analyses. On the other hand, almost one quarter of the ALF patients are transplanted [123] so an unfair bias would be introduced in the analysis if these patients were excluded. Most studies have opted to include transplanted patients and consider them together with nonsurvivors [7,150], in contrast to the spontaneous survivors who do not undergo transplantation.

The prognosis of ALF has improved over time [151] and the studies listed in Table 5 span over 4 decades, from the 1970s and 1980s (2-97) to the 2000s [7]. Therefore, it is interesting that the predictive values (and sensitivity and specificity) are so relatively unchanged over the years. The robustness of such prognostic tests could also explain why the King’s College Hospital criteria are still so widely used, even though an attempt to improve the criteria with the inclusion of arterial lactate has recently been suggested [140]. ALF is such a complex disease, so it is not unexpected that a single prognostic test cannot be perfectly accurate. Therefore, in the future, total and free Gc-globulin levels should be tested with prognostic markers that display other aspects of liver function, e.g. liver regeneration (alpha-feto protein), hepatocyte necrosis (ferritin), or features of infections (SIRS).

### MULTIPLE ORGAN FAILURE IN ACUTE LIVER FAILURE

A failing liver is the initial event in ALF that, by definition, leads to hepatic encephalopathy. However, other organs may also fail contributing to the morbidity and mortality and making ALF such a challenging condition [152-154]. Sepsis or evidence of the systemic inflammatory response syndrome (SIRS) are probably of paramount importance for the development of multiple organ failure (MOF) in ALF [155] and may also lead to worsening of hepatic encephalopathy [156]. Patients with ALF often develop renal failure, arterial hypotension, severe infections, and occasionally pulmonary dysfunction [151]. However, the most feared complication in ALF is the development of cerebral edema and intracranial hypertension where cerebral inaccretion is imminent [157,158]. Multiple organ dysfunction (MOD) is perhaps a better term than MOF since MOD describes a continuum of dysfunction whereas MOF is a dichotomous evaluation with fewer nuances [159].

The pathogenesis of MOF in ALF is not nearly clarified. Cyto-

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**Table 4. Clinical studies on serum Gc-globulin in acute liver failure (ALF). Levels are given as ratios compared to normal values.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Description</th>
<th>N</th>
<th>T-Gc</th>
<th>CR</th>
<th>F-Gc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al (109)</td>
<td>1985</td>
<td>ALF</td>
<td>14</td>
<td>0.27</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Goldschmidt-Ci. et al (96)</td>
<td>1985</td>
<td>ALF</td>
<td>11</td>
<td>0.31</td>
<td>1.9</td>
<td>NA</td>
</tr>
<tr>
<td>Goldschmidt-Ci. et al (101)</td>
<td>1988</td>
<td>ALF</td>
<td>7</td>
<td>0.38</td>
<td>NA</td>
<td>0.38</td>
</tr>
<tr>
<td>Schiodt et al (1)</td>
<td>1995</td>
<td>ACM OA/extreme</td>
<td>8</td>
<td>0.31/0.25</td>
<td>2.2/5.4</td>
<td>NA</td>
</tr>
<tr>
<td>Schiodt et al (97)</td>
<td>1995</td>
<td>ALF (mainly ACM)</td>
<td>47</td>
<td>NA</td>
<td>NA</td>
<td>0.17</td>
</tr>
<tr>
<td>Schiodt et al (2)</td>
<td>1996</td>
<td>ALF</td>
<td>94</td>
<td>0.35</td>
<td>3.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Wians et al (106)</td>
<td>1997</td>
<td>ALF</td>
<td>20</td>
<td>0.49</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Schiodt et al (6)</td>
<td>2001</td>
<td>ACM OA/extreme</td>
<td>15</td>
<td>0.29</td>
<td>4.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Schiodt et al (7)</td>
<td>2005</td>
<td>ALF</td>
<td>182</td>
<td>0.26</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Antoniades et al (110)</td>
<td>2005</td>
<td>ALF</td>
<td>53</td>
<td>NA</td>
<td>NA</td>
<td>0.12</td>
</tr>
<tr>
<td>Schiodt et al (148)</td>
<td>2005</td>
<td>ALF</td>
<td>178</td>
<td>0.17</td>
<td>NA</td>
<td>0.25</td>
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**Table 5. Prognostic value of Gc-globulin in acute liver failure.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Location</th>
<th>N</th>
<th>Patients</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al (97)</td>
<td>London</td>
<td>Admission</td>
<td>34</td>
<td>47</td>
<td>68</td>
<td>68</td>
<td>59</td>
<td>76</td>
</tr>
<tr>
<td>(Free Gc)</td>
<td></td>
<td>Day 2</td>
<td>27</td>
<td></td>
<td>100</td>
<td>85</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Schiodt et al (2)</td>
<td>Copenhagen</td>
<td>100</td>
<td>59</td>
<td>N-ACM</td>
<td>79</td>
<td>60</td>
<td>73</td>
<td>68</td>
</tr>
<tr>
<td>(Total Gc)</td>
<td></td>
<td>ACM</td>
<td>18</td>
<td></td>
<td>100</td>
<td>53</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Schiodt et al (7)</td>
<td>US multi-</td>
<td>80</td>
<td>106</td>
<td>N-ACM</td>
<td>85</td>
<td>43</td>
<td>65</td>
<td>69</td>
</tr>
<tr>
<td>(Total Gc)</td>
<td>center</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N-ACM = nonacetaminophen etiology. PPV = positive predictive value of a test. NPV = negative predictive value of a test. Sensitivity = proportion of positives (here: nonsurvivors or transplanted patients) correctly identified by the test. Specificity = proportion of negatives (here: nonsurvivors or transplanted patients) correctly identified by the test.

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Figure 6. Admission total and free Gc-globulin concentrations and relationship to development of organ failures in patients with grade III and IV acute liver failure. Patients who developed cardiovascular failure, intracranial hypertension, and infections had significantly lower Gc-globulin levels compared to those who did not develop these complications.

*) p < 0.01. **) p < 0.001.

Figure 7. The relationship between admission levels of total and free serum Gc-globulin values and number of organ failures in patients with acute liver failure and hepatic coma grade III or IV (A and C). B and D: the quintiles of total and free Gc-globulin vs. number of organ failures. Spearman's rank correlation coefficient was –0.42 (total Gc) and –0.46 (free Gc), P < 0.005 for both.

Tokines (e.g., IL-6, IL-1, and TNF), endotoxemia, or ischemia have been suggested to be important variables [160]. Lack of Gc-globulin could be one of the factors contributing to the development of MOF in ALF, since this could lead to actin-induced thrombosis resulting in tissue hypoxia, a frequent complication of ALF [161-163]. Seventy-nine patients with ALF and peak hepatic encephalopathy grade III/IV (a subset of the patients reported in study [2]) were studied with respect to admission levels of Gc-globulin and the development of organ failure [3]. The most common organ failure was pulmonary failure, followed by renal failure, infection, cardiovascular failure, and intracranial hypertension. Total and free Gc-globulin levels were significantly lower in patients developing infection, cardiovascular failure, or intracranial hypertension, whereas levels did not differ among patients with or without pulmonary or renal failure (Figure 6).

Patients with Gc-globulin values in the first quintile (lowest 20%) had almost 3 times as many organ failures as patients with values in the fifth quintile (Figure 7). Sixty-five percent of the patients developed MOF, defined as two or more organ failures (in addition to the hepatic failure and the presence of hepatic encephalopathy). These patients had lower total and free Gc-globulin than patients without MOF.

Is lack of Gc-globulin pathogenetically involved in the development of MOF? It seems highly unlikely that a single mediator should be responsible for all the profound disturbances seen in ALF. Rather, lack of Gc-globulin may be part of the explanation, together with mediators such as TNF, IL-1, IL-6, nitric oxide, and important cells like Kupffer cells, macrophages, endothelial cells, and the immunologic system [160]. Reduced Gc-globulin levels may be suggested to influence the course of illness in two ways: by the formation of (local) ischemia caused by actin thrombi formation, or by increasing the susceptibility to infection via a decrease in the nonspecific immune functions of Gc-globulin (Table 1). Capillary obstruction may be caused by cellular debris (actin, collagen) from the failing liver. Bihari et al demonstrated tissue hypoxia to occur in patients with grade III and IV ALF, evidenced by hyperlactatemia and metabolic acidosis [161,162]. Micrvascular disturbances are apparently the main cause of tissue hypoxia, perhaps developing because of arteriovenous shunting [162], reflected hemodynamically as reduced systemic vascular resistance and decreased oxygen extraction ratio [161]. However, lactic acidosis may also stem from accelerated glycolysis [153]. It remains to be studied if actin-containing thrombi are a pathologic feature of ALF.

In conclusion, it is not proven that lack of Gc-globulin/actin toxicity contributes to the development of MOF in ALF. However, even in these extremely sick patients, Gc-globulin levels clearly reflect the
risk of organ failures. Admission Gc-globulin concentrations can therefore indicate if subsequent MOF develops.

**GC-GLOBULIN KINETICS IN LIVER DISEASE**

Gc-globulin is almost entirely synthesized in the liver although smaller amounts of Gc-globulin mRNA are also expressed in the kidney, the testis, and the abdominal fat in rats [26]. In normal individuals, Gc-globulin kinetics was studied by injection of radio-labeled Gc-globulin [125]-labeled Gc) [34]. They found that a three-pool model could best explain Gc-globulin kinetics, pool 1 being plasma, pool 2 the extravascular, extracellular compartment, and pool 3 the intracellular compartment. The total exchangeable Gc-globulin was 2.89 gram and the production rate was 0.69-0.93 g/day with a mean of 0.80 g/day [34].

Protein turnover is generally decreased with advanced liver disease, most so in patients with hepatic coma where protein synthesis is only one third to one half of that observed in normal individuals [164].

Guha et al. studied the regulation of Gc-globulin expression in vitro in Hep3B hepatocytes and found that interleukin-6 and dexamethasone increased Gc mRNA and secreted protein by twofold whereas TGFβ decreased it by fivefold [165]. IL-1 and TNF did not affect Gc-globulin expression significantly. It is known that plasma levels of IL-6 (along with IL-1 and TNF) are increased in acute liver failure [166]. In an animal study, Gc-globulin mRNA expression increased slightly after inflammation whereas partial hepatectomy lead to a decrease in mRNA levels [167].

In study [5], Gc-globulin kinetics were studied in 22 patients with acute and chronic liver disease, all undergoing liver vein catheterization. Total and free Gc-globulin concentrations were lowest in patients with ALF, and patients with chronic liver disease had concentrations approximately 2-fold higher than in ALF (Figure 8), in keeping with the results reported elsewhere in this thesis. No difference in Gc-globulin concentrations in the hepatic vein, the artery, and the central vein was detected even though Gc-globulin is presumed to be almost entirely synthesized in the liver. This apparent discrepancy can be rather easily explained by the inaccuracy of the analytical method.

Most patients with hepatic encephalopathy also underwent high volume plasmapheresis (exchange of 8-10 liters of plasma), a treatment option that may improve survival in some patients with ALF serving as a bridge to urgent liver transplantation [113;168]. An estimate of Gc-globulin production and half-life could be made in these patients assuming a single compartment model and a volume of distribution (Vd) of 6 litres (Figure 8). The Gc-globulin production rate was 4.1 ± 1.3 mg/min – 7-fold higher than the production rate reported in healthy adults in the literature [34]. This surprising observation indicates a high priority for Gc-globulin in the necrotic liver – as opposed to the conditions after partial hepatectomy [167] – and protection against actin toxicity seems like an obvious explanation for this high priority, since actin release from necrotic hepatocytes is so abundant. Also, Gc-globulin's immune functions may be needed in ALF and acute on chronic liver disease, both conditions being characterized by a high proportion of infections [151;169]. Still, Gc-globulin concentrations were low despite the Gc-globulin production increase, and this was due to a shorter than normal half-life of Gc-globulin [5]. The Gc-globulin:actin complex has a much shorter half-life than uncomplexed Gc-globulin [36;59] and the observed decrease in Gc-globulin's half-life is possibly due to increased actin complexing. However, Gc-globulin could also have been consumed as part of its function as a precursor of macrophage activating factor [170;171] or activation of killer cells [83]. A possible bias in our study [5] was the fact that we compared our results in liver patients with normal subjects reported in the literature rather than performing the same measurements in normal subjects. However, the results were so striking that it is very unlikely that this would have changed the conclusions of the study.

Bound Gc-globulin was constant and within normal range in all patients and also before and after high volume plasmapheresis, in keeping with the results reported in [6]. We speculate that uptake and degradation of the Gc-globulin:actin complexes are regulated by bound Gc-globulin concentrations, even in situations of severe liver damage, by a receptor mechanism (Figure 9). The complexes are taken up in sinusoidal endothelial cells or Kupffer cells [58;59] and these cell lines may not suffer as much as hepatocytes during liver injury [172;173] which could mean that the proposed regulatory mechanism is still intact in ALF and severe chronic liver disease. However, proof for this hypothesis is lacking. Also, this hypothesis

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**Figure 8.** Total, free, and bound Gc-globulin levels and complex ratio in patients with acute liver failure (FHF), acute on chronic liver disease (AOC), and chronic liver disease without coma (CLD) compared with normal controls (Nor).

**Figure 9.** Model for estimation of production rate and half-life of Gc-globulin. A: hepatic production rate of Gc-globulin. Plasma infusion rate during high volume plasmapheresis. C(t): concentration of Gc-globulin at any given time. k1: rate constant for plasma removal during plasmapheresis. k2: rate constant for Gc-globulin clearance. Vd: volume of distribution of Gc-globulin.
may be challenged by the finding of very low (<5%) actin complex ratio levels in one study using crossed immunoelectrophoresis [100] rather than rocket immunoelectrophoresis. This is also what should be expected since the half-life of the Gc-globulin:actin complex is so much shorter than that of uncomplexed Gc-globulin [34,36] — unless of course the proposed hypothesis is correct. Further studies using several methods of analysis in the same serum samples should elucidate these points.

**CHRONIC LIVER DISEASE AND LIVER TRANSPLANTATION**

Chronic liver disease may arise from a number of etiologies. However, once cirrhosis develops symptomatology and clinical findings are very similar among the patients with the risk of decompensation in the form of variceal bleeding, ascites, hepatic encephalopathy, or hepatic encephalopathy.

Gc-globulin levels in chronic liver disease without cirrhosis have been reported in 5 studies (Table 6) [16,96;104;105;109]. Serum Gc-globulin was normal in 2 of those studies and below normal in the remaining 3; in 2 of them levels were one half of normal. The complex ratio was only measured in one study where Goldschmidt-Clermont et al found complex ratio levels to be above normal [96].

Nine studies have reported on Gc-globulin levels in cirrhotic patients (Table 6) [4;5;20;63;103;105;107;174;175]. Total Gc-globulin concentrations were reduced in all studies, to between 45% and 92% of normal levels in compensated cirrhosis and to between 33% and 69% in decompensated cirrhosis. Complex ratio was measured in 2 studies [4;5] and was found to be increased in one of those studies [5]. Free Gc-globulin levels were reported in two studies [4,5] and were below normal range in both of them, in parallel with total Gc-globulin concentrations (Table 6).

Liver transplantation is required for some patients with decompensated cirrhosis [176]. After liver transplantation, Gc-globulin genotype seems to convert to that of the donor [177]. Gc-globulin and complex ratio in patients with end-stage liver disease before and after liver transplantation were reported in one study [4]. A minority of the patients had normal Gc-globulin levels before transplantation. In this group Gc-globulin levels remained normal after transplantation (Figure 11). The majority of the patients had subnormal Gc-globulin levels before transplantation. In this group Gc-globulin levels gradually increased in most patients after transplantation (Figure 11). This course paralleled that of the increase of the prothrombin index but was in contrast to the continuous decrease in albumin levels (Figure 12). So even though albumin and Gc-globulin from $= Gc$-globulin = Actin.

Figure 10. A schematic view of the hypothesized results of hepatic necrosis on Gc-globulin and actin complex ratio. Total and free Gc-globulin concentrations decrease significantly following hepatocyte necrosis, whereas bound (actin-complexed Gc-globulin) remains constant and normal due to a proposed regulatory mechanism in endothelial cells that is intact even in liver failure.

Table 6. Clinical studies on serum Gc-globulin in chronic liver disease. Levels are given as ratios compared to normal values.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Cirrhosis Patients</th>
<th>N</th>
<th>T-Gc</th>
<th>CR</th>
<th>F-Gc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barragry et al (16)</td>
<td>1978</td>
<td>No CLD (PBC, ALD, CAH)</td>
<td>45</td>
<td>0.78</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bikle et al (104)</td>
<td>1984</td>
<td>No ALD</td>
<td>25</td>
<td>0.47</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lee et al (109)</td>
<td>1985</td>
<td>No CLD (PBC, ALD, CAH)</td>
<td>49</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Goldschmidt-Cl. et al (96)</td>
<td>1985</td>
<td>No CAH</td>
<td>7</td>
<td>0.52</td>
<td>1.6</td>
<td>NA</td>
</tr>
<tr>
<td>Diamond et al (105)</td>
<td>1989</td>
<td>No CLD</td>
<td>54</td>
<td>1.06</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bouillon et al (63)</td>
<td>1977</td>
<td>Yes Cirrhosis</td>
<td>16</td>
<td>0.8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Brown et al (20)</td>
<td>1979</td>
<td>Yes Cirrhosis</td>
<td>37</td>
<td>0.77</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Walsh et al (103)</td>
<td>1982</td>
<td>Yes Cirrhosis</td>
<td>4</td>
<td>0.65</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Constans et al (19)</td>
<td>1983</td>
<td>Yes Alcoholic cirrhosis</td>
<td>17</td>
<td>0.62</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bouillon et al (174)</td>
<td>1984</td>
<td>Yes Cirrhosis</td>
<td>32</td>
<td>0.92</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Masuda et al (175)</td>
<td>1989</td>
<td>Yes Cirrhosis</td>
<td>8</td>
<td>0.67</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diamond et al (105)</td>
<td>1989</td>
<td>Yes Cirrhosis</td>
<td>53</td>
<td>0.88</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Schiedt et al (4)</td>
<td>1999</td>
<td>Yes ESLD</td>
<td>17</td>
<td>0.69</td>
<td>1.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Schiedt et al (5)</td>
<td>2001</td>
<td>Yes Cirrhosis</td>
<td>8</td>
<td>0.45</td>
<td>1.9</td>
<td>0.35</td>
</tr>
</tbody>
</table>

ALD = alcoholic liver disease. CAH = chronic active hepatitis. CLD = chronic liver disease. CR = complex ratio. ESLD = end-stage liver disease. F-Gc = free Gc-globulin. HE = hepatic encephalopathy. PBC = primary biliary cirrhosis. T-Gc = total Gc-globulin.
ically in decompensated cirrhosis. Future studies should elucidate if this decrease is caused by a fall in Gc-globulin production or an increased Gc-globulin clearance - or both. Gc-globulin concentrations normalize in most patients within the first 2 weeks following liver transplantation, in contrast to albumin concentrations, indicating a higher priority for Gc-globulin.

OTHER ASPECTS OF THE EXTRACELLULAR ACTIN-SCAVENGER SYSTEM IN DISEASE

Serum Gc-globulin levels have been studied in other conditions than liver diseases (Table 7).

The degree of reduction of Gc-globulin levels in disease seems to correlate with both the amount of necrosis and the cytokine response (systemic inflammatory response syndrome, SIRS), since the lowest levels have been observed in septic shock [180], a condition characterized by widespread necrosis and hyperactivation of SIRS [192-194]. Moderate reductions of serum Gc-globulin have been observed in multiple trauma [163;185-187], in which SIRS is less activated than in septic shock [194]. The decrease in Gc-globulin concentrations in trauma can be observed as early as 45 minutes after injury [185] and Gc-globulin levels have prognostic value also in this condition [186]. The reduced levels in nephrotic syndrome are caused by urinary loss of Gc-globulin [184].

Interestingly, pregnancy (especially late pregnancy) seems to induce an increased synthesis of Gc-globulin [16;63;96;100;103;104], possibly due to increased estrogen levels, as estrogen therapy causes increased Gc-globulin levels [16]. For unknown reasons short bowel syndrome patients have also increased serum Gc-globulin values (Schmidt et al., data not published).

The other protein of the extracellular actin scavenger system, gelsolin, has been studied in a number of diseases (Table 8).

All the diseases mentioned in Table 8 - except cancer - involve acute cellular necrosis. Serum levels of gelsolin seem to correlate with the degree of disease severity and the lowest levels have been observed in ALF. It is not known if the reduction of serum gelsolin concentrations is related solely to an increased consumption of gelsolin due to actin scavenging or maybe also to decreased gelsolin production.

Thus, in diseases and conditions involving tissue necrosis or tissue injury the two components of the extracellular actin scavenger system, Gc-globulin and gelsolin, are invariably affected and serum levels of the 2 proteins are reduced, most so in patients with ALF or septic shock. It remains to be studied if this reduction may be pathogenetically involved in the diseases. One very recent study (208) suggests so, since infusion of recombinant gelsolin to endotoximic mice improved survival significantly.

PERSPECTIVES:

The papers described in this thesis confirm that the actin scavenger...
system is significantly affected in liver disease. Serum levels of Gc-globulin are often markedly reduced and the reduction correlates with the severity and the acuity of the disease, since Gc-globulin concentrations were lowest among patients with ALF, a condition characterized by massive hepatic necrosis. This supports that Gc-globulin is consumed upon tissue necrosis with concomitant actin release. Gc-globulin levels could also be reduced if Gc-globulin were used in its known nonspecific immune functions. This seems very probable since liver diseases are characterized by a high proportion of infections. Future research should try to elucidate Gc-globulin's role in infected liver patients in more detail.

Admission serum Gc-globulin was demonstrated to be of equal value as the King's College Hospital criteria in determining outcome in ALF. The advent of rapid methods of analysis (e.g., immunonephelometry and ELISA techniques) suggests that measurement of serum Gc-globulin could have value for the clinicians taking care of patients with ALF, acetaminophen intoxication, or acute on chronic liver disease. Also, there are a number of severe hepatic conditions with a high mortality where the role of Gc-globulin has not been studied yet, e.g., alcoholic hepatitis or spontaneous bacterial peritonitis.

The question is really if Gc-globulin - or lack of Gc-globulin - is in any way related to the pathogenesis of severe liver failure. Support for this hypothesis can be found in the observation that even in patients with the deepest grades of hepatic encephalopathy admission Gc-globulin levels had a highly significant predictive value for the later development of organ failure and MOF. Thus, lack of Gc-globulin with the severity and the acuity of the disease, since Gc-globulin concentrations were lowest among patients with ALF, a condition characterized by massive hepatic necrosis. This supports that Gc-globulin is consumed upon tissue necrosis with concomitant actin release. Gc-globulin levels could also be reduced if Gc-globulin were used in its known nonspecific immune functions. This seems very probable since liver diseases are characterized by a high proportion of infections. Future research should try to elucidate Gc-globulin's role in infected liver patients in more detail.

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diagram. The total Gc-globulin concentration in a sample was calculated by plotting the natural logarithm (ln) versus the rocket height of the sample with added actin in the diagram and using the upper standard curve. A perpendicular line from this point on the Standard + actin curve was drawn. Next, the ln (rocket height of the sample with no added actin) was plotted in the diagram, and the junction between this value and the perpendicular of the Gc-globulin + actin was noted. The complex ratio was calculated as the ratio y/(y + x). A semiautomated computer program (in the software program Lotus 1-2-3 version 2.3 for DOS) was constructed to calculate serum Gc-globulin and complex ratio. The two standard curves were calculated using linear regression analysis. Table 9 shows the results of fifty consecutive standard curves. Both standard curves had mean and median R² values over 0.99, highest for the standard curve with actin. The slope of the curve was steeper for the lower standard curve, in accordance with the original description (96). Standard error for the slope was in the 2 to 5 per cent range for both curves, whereas standard errors of Y were 4 % and 8 %, respectively. Repeated analyses of a given sample yielded a 6 % difference of the calculated serum Gc-globulin value (coefficient of variance), and a little higher difference for the calculated complex ratio.

**SUMMARY**

**ENGLISH**

This Doctoral thesis is based on 7 previously published papers and reports on the role of the actin-scavenger Gc-globulin in acute and chronic liver diseases. Gc-globulin is synthesized in the liver and is a multifunctional protein; however, its main physiologic function is presumably actin binding and actin scavenging. Actin is a major cellular protein released during cell necrosis that may cause fatal formation of actin-containing thrombi in the circulation if the actin scavenging capacity of Gc-globulin is exceeded.

In my studies, I found serum Gc-globulin levels to be reduced in liver disease, most so in patients with acute liver failure (ALF). In patients admitted with acetaminophen (paracetamol) overdose, Gc-globulin concentrations were lower in patients with hepatic encephalopathy than in those without and the levels nadired at approximately 60-72 hours after acetaminophen ingestion, corresponding with the peak in aminotransferase levels (and thus, hepatic necrosis).

In patients with ALF, admission Gc-globulin was significantly lower in 47 nonsurvivors than in 30 survivors, 26% and 46% of normal, respectively (P<0.001). The predictive value of outcome using a Gc-globulin cutoff level of 100 mg/L equaled that of the internation X-ratio test and was 95% for both bound and free Gc-globulin after acetaminophen overdose. Liver Transpl Surg 1999; 5:310-317.


Table 9. The results (mean, median, range) of standard curve linear regression analysis from fifty consecutive rocket immunoelectrophoresis plates, analyzed between April and August 1995. The equation for a line is: y = constant + αx. Values for standard curves without and with actin were compared with Mann-Whitney’s rank sum test (Normality test failed for all groups). SE = standard error.

<table>
<thead>
<tr>
<th>Standard curve without actin</th>
<th>CONSTANT</th>
<th>SE of y</th>
<th>R²</th>
<th>α</th>
<th>SE of α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>-0.931</td>
<td>0.081</td>
<td>0.991</td>
<td>0.958</td>
<td>0.051</td>
</tr>
<tr>
<td>median</td>
<td>-0.862</td>
<td>0.078</td>
<td>0.991</td>
<td>0.945</td>
<td>0.049</td>
</tr>
<tr>
<td>range</td>
<td>(-2.497,-0.181)</td>
<td>(0.047-0.143)</td>
<td>(0.980-0.998)</td>
<td>(0.808-1.246)</td>
<td>(0.030-0.090)</td>
</tr>
</tbody>
</table>

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