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Platelets

Anti-Hepatitis C Virus Serology in Immune Thrombocytopenia: A Retrospective Analysis in 101 Patients

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Hepatitis C virus (HCV), an RNA virus, is known to be the major cause of post-transfusion non-A, non-B hepatitis. HCV can induce several expressions of autoimmunity, including both serological abnormalities and clinical disorders. The relationship between the HCV infection and anti-platelet autoimmunity has been occasionally described, but is still far from well-defined. We retrospectively analysed 101 serum specimens, collected between 1988 and 1994, from patients with immune thrombocytopenia (ITP) for the presence of anti-HCV antibodies. Eighty-seven patients were classified as having idiopathic, and 14 secondary ITP (4 systemic lupus erythematosus, 9 non-Hodgkin's lymphoma and 1 Evan's syndrome).

Anti-HCV antibodies were determined by second generation tests (ELISA + RIBA). A specimen was considered positive for HCV antibodies in the presence of ELISA reactivity (sample optical density/cut-off > 1.00) accompanied by RIBA reactivity to at least one HCV specific antigen. 20 sera (20%) were positive, with a prevalence higher in secondary than in idiopathic ITP (43% *vs.* 16%, $p < 0.05$). No differences were found between anti-HCV positive and negative patients regarding gender, platelet count, platelet associated immunoglobulins, hepatitis B virus serology and liver enzyme profile. On the contrary, mean age was higher in the HCV positive *vs* HCV negative ones (58 ± 18 SD *vs.* 44 ± 20 yrs, $p < 0.01$), in keeping with the increasing prevalence of HCV infection with ageing. HCV positive patients, showed a poor response to treatment (platelet count lower than 50,000/μl after conventional medical therapy for immune thrombocytopenia) compared to anti-HCV negative ones, (50% *versus* 7.3%, $p < 0.001$). When we excluded patients who were exposed to risk factors for HCV infection after ITP diagnosis and before the serum collection, the prevalence of anti-HCV antibodies was not very different (17.6%) from that found in the series as a whole (19.8%). Our results seem to indicate that HCV infection may play a role in triggering several cases ITP, and moreover might constitute a negative prognostic factor for therapy response.

Keywords: Hepatitis C, immune thrombocytopenia

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INTRODUCTION

Viral infections are often associated with thrombocytopenia and hemorrhagic symptoms. Virus-induced thrombocytopenia is a combination of direct and indirect mechanisms; including (a) disturbance of megakaryocytopoiesis by virus replication in the megakaryocytes which appear to be a hospitable environment for viruses, (b) microangiopathic consumption of platelets, (c) phagocytosis of platelets sensitized by immune-complexes, and (d) induction of an autoimmune response [1]. Viruses can promote autoimmunity through the production of anti-idiotype antibodies, enhanced expression of the major histocompatibility complex (MHC) Class I° and II° molecules, disturbances in the hosts immune response, changes in endogenous antigen and molecular mimicry [2].

Recent data have evidenced the existenced of important homologies between the main platelet surface autoantigen, GP IIb-IIIa, and some proteins of at least eight common human viruses, *i.e.*, Herpes Simplex, Varicella Zooster, Epstein-Barr, Adenovirus, Cytomegalovirus, Measles, Mumps and Rubella [3].

HCV, an RNA virus, is known to be the major causative agent of post-transfusion non-A, non-B hepatitis. HCV can induce several expressions of autoimmunity, including serological abnormalities *i.e.*, rheumatoid factor [4], and anti-nuclear [5], anti smooth muscle [6], anti-LKM-1 [7], anti-neutrophil [8] and anti-cardiolipin [9] antibodies, as well as extra-hepatic clinical disorders *i.e.*, mixed cryoglobulinemia [10], porphyria cutanea tarda [11], lichen planus [12], sicca like syndrome [13], autoimmune thyroid disease [14] and glomerulonephritis [15].

The association between HCV infection and immune thrombocytopenia (ITP) has been occasionally described [16, 17], but its real frequency is not yet well defined. Anti-HCV antibodies were detected in 11% of patients with ITP [18]; moreover patients with chronic hepatitis C presented high level of platelet associated immunoglobulins, and were carriers of HCV-RNA [19].

We analysed retrospectively 101 serum specimens from patients with ITP for the presence of anti-HCV antibodies. Our major aim was to establish the prevalence of anti-HCV antibodies independently from any overt risk of viral transmission occurring after the onset of the platelet disorder.

MATERIALS AND METHODS

One hundred-one sera, collected in the period 1988-1994 from consecutive patients with chronic ITP (28 males and 73 females; mean age, 47 ± 22SD years; range 5-88 years), were tested for the presence of anti-HCV antibodies. The diagnostic criteria for chronic ITP were: (a) platelet count lower than 100,000/μl for at least 6 months; (b) normal or increased number of megakaryocytes on bone marrow examination; (c) exclusion of hypersplenism and/or splenomegaly (white and red cell count, spleen ultrasound and/or scintigraphy); (d) no recent use of drugs ingestion known to induce thrombocytopenia; (e) exclusion of sepsis and/or microangiopathy, and (f) exclusion of familial inheritance of thrombocytopenia. Platelet associated IgG (PAIgG) were assayed as previously described [20], but the presence of increased levels of PAIgG was not considered a major diagnostic criterion for ITP.

Eighty-seven patients were classified as having "idiopathic" ITP and 14 had "secondary" ITP (4 cases of systemic lupus erythematosus, 9 of lymphoma and 1 of Evan's syndrome). The response to conventional therapy (Prednisone 1 mg/Kg/d; Danazol 400 mg/d or high-dose iv. IgG 400 mg/Kg/d × 5 days) was judged good or poor, when the increase in platelet count was respectively higher or lower than 50,000/μl.

The following risks of HCV infection were gathered from the clinical records of the patients: (a) exposure to blood products before

the introduction of anti-HCV screening of blood donors in Italy (Autumn, 1989), and to plasma derivatives (iv. IgG); (b) major surgery; (c) sexual promiscuity, and (d) iv. drug addiction.

All the sera specimens were screened for hepatitis B virus (HBV) and HIV1/2 serologic markers, and the liver enzyme profile was determined. HBcAb positivity was considered as a marker of previous HBV infection [21]; liver disease was presumed when high ALT levels (>2 upper normal limit) persisted for at least four months.

Anti-HCV antibodies were assayed by a second generation ELISA (Ortho, HCV ELISA test system 2nd generation, Raritan, NJ, USA). Serum specimens were judged reactive when the optical density/cut off ratio was >1.00. As a supplemental test, a recombinant immunoblot assay (Chiron RIBA HCV 2.0, Emeryville, Ca, USA) was performed on the reactive serum specimens. All anti-HCV tests were conducted according to the manufacturer's instructions. An anti-HCV positive result was considered positive when RIBA-II showed reactivity (intensity 1+ or more) against at least one HCV specific antigen (c100-3, 5-1-1, c33-c and c22-3).

Statistical analysis was performed by the X^2 test and Student's t-test; $p < 0.05$ was considered significant.

RESULTS

All the patients were HIV-1/2 seronegative.

As shown in Table I, anti-HCV antibodies were detected by ELISA in 20/101 sera (19.8%), and the prevalence was higher in secondary ITP (6 out of 14 sera, 43%), compared to primary ITP (14 out 87 sera, 16%, $p < 0.05$). By means of the supplemental RIBA test, all positive sera on ELISA showed reactivity against core antigen c22-3 (100%) , 13 against c-33c (65%), 9 against c100-3 (45%), and 8 against 5-1-1 (40%). Gender distribution, platelet count, PAIgG positivity, prevalence of abnormal liver enzyme profile and HBV serology were similar in both HCV positive and negative patients (Tab. I). However, HCV positive patients were older than the HCV negative ones (mean age 58 ± 4 SE years *vs.* 44 ± 2 years, $p < 0.01$), and showed a poorer response to conventional treatment for ITP (good response 50% *vs.* 92.7%, $p < 0.001$).

In reference to risk factors for HCV, all patients denied sexual promiscuity and iv. drug addition. As in Table II, 8 of 20 (40%) HCV positive, and 25 of 81 (31%) HCV negative patients were exposed to risk factors for HCV infection (blood transfusion and/or major surgery) after ITP diagnosis and before sampling for the anti-HCV assay (Columns A2 and B2). Conversely, 8 of 20 (40%) HCV positive and 30 of 81 (37%) HCV negative patients were exposed to risk factors before ITP diagnosis (Columns A1 and B1). Moreover 4 of 20 (20%) HCV positive, and 26 of 81 (32%) HCV negative patients were not exposed to risk (Columns A3 and B3). Therefore the real prevalence of anti-HCV positivity in ITP patients, following correction on the basis of risk factors for viral infections, was 17.6% (12 of 68 patients: Columns $(A1 + A3)/(A1 + A3) + (B1 + B3)$.

DISCUSSION

It is well known that viral infections can induce thrombocytopenia by multiple mechanisms. A direct effect of the virus on megakaryocytes and platelets is usually responsible for an acute, self-limited form, while indirect induction of the autoimmune process can trigger chronic immunomediated thrombocytopenia [2].

A thrombocytopenia due to a combination of direct and indirect effect of virus is best exemplified by the thrombocytopenia observed during HIV infection [22–24].

The occurrence of thrombocytopenia during hepatotropic virus infection is not common. In anedoctal cases of hepatitis A virus infection, thrombocytopenia has been associated with

TABLE I Main features of 101 patients affected with immune thrombocytopenia according to the anti-hepatitis C virus (HCV) serology

	Anti-HCV + ve		Anti-HCV − ve
Total	19.8%		80.2%
Idiopathic ITP	16%		84%
Secondary ITP	**43%**	**($p < 0.05$)**	**57%**
Age (years)	**58 ± 4***	**($p < 0.01$)**	**44 ± 2***
Male	30%		27%
Female	70%		73%
Platelet count ($\times 10^9$/l)	55 ± 13*		59 ± 6*
PAIgG + ve	9/20 (45%)		46/81 (57%)
HBV+ve	9/20 (45%)		23/81 (28%)
Abnormal liver enzymes	4/20 (20%)		6/81 (7%)
Response to Therapy for thrombocytopenia			
-poor	**50%**		**7.3%**
-good	50%	**($p < 0.001$)**	92.7%

*mean ± standard error.

TABLE II Association between anti-HCV serology and risk factors for HCV infection in 101 patients affected with immune thrombocytopenia

Risk factors	Anti-HCV + ve			Anti-HCV − ve		
	A1 exposure before ITP	A2 exposure after ITP	A3 no exposure	B1 exposure before ITP	B2 exposure after ITP	B3 no exposure
Blood derivatives	7	7	0	6	14	0
Major surgery	1	1	0	24	11	0
Total patients	8	8	4	30	25	26
Percentage	40%	40%	20%	37%	31%	32%

Anti HCV seroprevalence in patients affected with ITP corrected by excluding patients having major risk factors for HCV infection after the diagnosis of ITP, according to the formula [Columns A1 + A3/Columns (A1 + A3) + (B1 + B3)] = 12/68 (18%).

either anti-platelet antibodies or immune-complexes [25], or a transient aplastic crisis [26] as also observed during parvovirus B19 infection [27]. Thrombocytopenia secondary to hepatitis B virus infection is more frequent, and usually attributed to hypersplenism [28], but some autoimmune mechanisms have also been evidenced [29].

While HCV infection appears clearly related to several autoimmune manifestations [30], the relationship between this infection and ITP is still doubtful. Nevertheless, there is some evidence that HCV may be involved in the pathogenesis of ITP. Indeed, HCV genomic sequences have been detected in platelets from patients with chronic hepatitis C [19, 31], and a higher prevalence of thrombocytopenia with increased PAIgG was found in patients with chronic hepatitis C, compared to patients with chronic hepatitis B [19].

In our retrospective analysis of 101 patients with ITP, we found a 19% prevalence of serum anti-HCV antibodies. This result agrees substantially with previous reports [18, 31, 32] in which prevalence ranged from 10% to 19%. Dine et Brahimi [33] reported a higher frequency of anti-HCV antibodies (39%); however, workers studied only virus-associated thrombocytopenias and likely overestimated the seroprevalence.

Although the frequency of anti-HCV antibodies in ITP was much lower than that demonstrated in the most well-recognized extrahepatic manifestation of HCV infection, *i.e.*, mixed cryoglobulinemia (> 80%), a prevalence of about 20%, as we documented, suggests that HCV

infection might be involved in several cases of ITP. Indeed, the anti-HCV seroprevalence in ITP is higher than the range (0–5%) observed in blood donors from our geographic area [34], in urban population of north–east Italy [35] and other immunomediated diseases, such as rheumatoid arthritis [36] and Sjogren's syndrome [37]. We also observed an high prevalence (32%) of anti-HBc antibodies especially in HCV positive patients; this could represent a co-infection due to similar risk factors. However, previous exposure to HBV in our patients was comparable to that reported in an agematched general population from the north of Italy [39]. Moreover the presence of anti-HBc antibodies were not statistically higher in HCV positive patients than that found in HCV negative ones.

Anti-HCV antibodies were significantly more frequent in patients with "secondary" than in those with "idiopathic" ITP. This figure may be explained by an high prevalence of non-Hodgkin's lymphoma in our patients with "secondary" ITP and by the well-recognized association between lymphoproliferative disorders and HCV infection [38]. Moreover, the HCV positive ITP patients were older than the HCV-negative ones, according to the age-related increasing risk of HCV infection [39].

A major limit of our retrospective study was the possibility that our patients might have been infected by HCV after ITP diagnosis. In fact patients with ITP have high risk of viral infection due to the exposure to blood derivatives and/or major surgery, *e.g.*, splenectomy [18]. However, when we excluded patients who were exposed to such risk factors after the diagnosis of ITP, the corrected prevalence of anti-HCV antibodies was 17%, which was not very different from the figure found in the series as a whole.

We thus suggest to include diagnostic testing for HCV infection in ITP patients, as this virus could play a role in triggering thrombocytopenia. This issue, moreover, might be relevant from a therapeutic viewpoint. Indeed, our results indicated that HCV positive ITP patients, as compared to HCV-negative ones, showed a worse response of thrombocytopenia to the conventional therapeutic regimens for ITP, including prednisone. In this regard it has been demonstrated that high-dose corticosteroids can increase HCV viremia [40]. Several reports have drawn attention to the beneficial effect of alpha-interferon in the extra-hepatic manifestation of HCV infection [41] as well as in patients with refractory ITP [42]. Therefore, in patients with HCV-associated ITP, alpha-interferon, could be proposed as a first line treatment of choice [43].

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References

[1] Zucker-Franklin, D. (1995). Platelets in viral infections. In: *"Immunopharmacology of platelets"*, Josef, M. (Ed.), Academic Press LTD, London, pp. 137–49.
[2] Kaplan, C., Morinet, F. and Cartron, J. (1992). Virus-induced autoimmune thrombocytopenia and neutropenia, *Semin. Hematol.*, **29**, 34–44.
[3] Boughton, B. J., Simpson, A. W., Bolt, C., Buchan, A. and McLeish, P. (1995). Platelet membrane glycoprotein IIb–IIIa has sequence homologies with human virus proteins and synthetic viral peptides inhibit anti-gp IIb–IIIa antibodies in autoimmune thrombocytopenic purpura, *Platelets*, **6**, 75–82.
[4] Clifford, B. D., Donahue, D., Smith, L. *et al.* (1995). High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C, *Hepatology*, **21**, 613–9.
[5] Richardet, J. P., Lons, T., Johanet, C. *et al.* (1994). Prèvalence et caractèristiques des anticorps anti-tissus au cours des hepatitis chroniques dues au virus de l'hepatite C, *Gastroenterol. Clin. Biol.*, **18**, 819–23.
[6] Abuaf, N., Lunel, F., Giral, P. *et al.* (1993). Non-organ specific autoantibodies associated with chronic C virus hepatitis, *J. Hepatol.*, **18**, 359–64.
[7] Lunel, F., Abuaf, N., Frangeul, L. *et al.* (1992). Liver/kidney microsome antibody type I and hepatitis C virus infection, *Hepatology*, **16**, 630–6.
[8] Warny, M., Brenard, R., Cornu, C., Tomasi, J. P. and Geubel, A. P. (1993). Anti-neutrophil antibodies in chronic hepatitis C and effect of alpha interferon therapy, *J. Hepatol.*, **17**, 294–300.
[9] Biron, C., Andreani, H., Blanc, P. *et al.* (1996). Antiphospholipid antibodies in patients with chronic hepatitis C virus infection or alcoholic liver disease, Preliminary results. *Ann. Med. Intern.*, **147** (suppl. 1), 48–9.

[10] Mazzaro, C., Tulissi, P., Moretti, M. *et al.* (1995). Clinical and virological findings in mixed cryoglobulinaemia. *J. Intern. Med.*, **238**, 153–60.
[11] Cribier, B., Petiau, P., Keller, F. *et al.* (1995). Porphyria cutanea tarda and hepatitis C viral infection. A clinical and virologic study, *Arch. Dermatol.*, **131**, 801–4.
[12] Sanchez-Perez, J., De Castro, M., Buezo, G. F. *et al.* (1996). Lichen planus and hepatitis C virus: prevalence and clinical presentation of patients with lichen planus and hepatitis C virus infection, *Br. J. Dermatol.*, **134**, 715–9.
[13] Jorgensen, C., Legouffe, M. C., Penney, P. *et al.* (1996). Sicca syndrome associated with hepatitis C virus infection, *Arthritis Rheum*, **39**, 1166–71.
[14] Marcellin, P., Pouteau, M. and Benhamou, J. P. (1995). Hepatitis C virus, alpha interferon therapy and thyroid dysfunction, *J. Hepatol*, **22**, 364–9.
[15] Stehman-Breen, C., Wilson, R., Alpers, C. E., Gretch, A. and Johnson, R. J. (1995). Hepatitis C virus associated glomerulonephritis, *Curr. Opin. Nephrol. Hypertens*, **4**, 287–94.
[16] Durand, J. M., Lefevre, P., Telle, H., Kaplanski, G., Quiles, N. and Soubeyrand, J. (1993). Thrombocytopenic purpura and hepatitis C virus infection, *Haematologica*, **78**, 135.
[17] Siciliano, R., Trovato, B. A., Seminara, G., Giustolisi, V. and Malaguarnera, M. (1995). Interferon or hepatitis C virus induced autoimmune aplastic anaemia and severe thrombocytopenia. A case report., *Ann. Ital. Med. Int.*, **10**, 193–4.
[18] Pawlotsky, J. M., Bouvier, M., Fromont, P. *et al.* (1996). Hepatitis C virus and autoimmune thrombocytopenic purpura, *J. Hepatol.*, **24**, 135–40.
[19] Nagagime, T., Ohtuka, T., Takehara, K., Arai, T., Takagi, H. and Mori, M. (1996). Thrombocytopenia associated with hepatitis C viral infection, *J. Hepatol.*, **24**, 135–40.
[20] Fabris, F., Cordiano, I., Steffan, A., Randi, M. L. and Girolami, A. (1992). Identification of antiplatelet autoantibodies by Western-blot in 45 patients with idiopathic thrombocytopenic purpura (ITP). *Haematologica*, **77**, 122–6.
[21] Krugman, S., Overby, L. R., Mushwar, I. K., Ling, C. M., Frosner, G. G. and Deinhardt, S. (1979). Viral hepatitis type B. Studies on natural history and prevention re-examined, *N. Engl. J. Med.*, **300**, 101–6.
[22] Morris, L., Distenfeld, A., Amorosi, E. *et al.* (1982). Autoimmune thrombocytopenic purpura in homosexual men, *Ann. Intern. Med.*, **96**, 714–7.
[23] Zucker-Franklin, D., Seremetis, S. and Zheng, Z. Y. (1990). Internalization of human mmunodeficiency virus type 1 and other retro-viruses by megakaryocytes and platelets, *Blood*, **75**, 1920–3.
[24] Karpatkin, S. and Nardi, M. (1992). Autoimmune anti-HIV-1 gp 120 antibody with anti-idiotype-like activity in sera and immune complexes of HIV-1 related immunologic thrombocytopenia, *J. Clin. Invest.*, **89**, 356–64.
[25] Kleinman, Y. and Friedman, G. (1982). Transient autoimmune thrombocytopenia associated with acute infectious hepatitis, *Hepato-gastroenterology*, **29**, 144–55.
[26] Ibarra, H., Zapata, C., Inostroza, J., Mezzano, S. and Riedeman, S. (1986). Immune thrombocytopenic purpura associated with hepatitis A, *Blut*, **52**, 371–5.
[27] Srivastava, A., Bruno, E., Briddel, R. *et al.* (1990). Parvovirus B19 induced perturbation of human megacaryocytopoiesis in vitro, *Blood*, **76**, 1997–2004.
[28] Gjerloff-Schmidt, A., Rasmussen, J. W., Bekker, C. and Rorbaek-Madsen, P. E. (1985). Kinetics and in vivo distribution of 111-In labelled autologous platelets in chronic hepatic disease: mechanism of thrombocytopenia. *Scand. J. Haematol.*, **31**, 39–46.
[29] Kelton, J. G., Carter, C. J., Rodger, C. *et al.* (1984). The relationship among platelet-associated IgG, platelet lifespan and reticuloendothelial cell function. *Blood*, **63**, 1434–8.
[30] Lunel, F. (1994). Hepatitis C virus and autoimmunity: fortuitous association or reality? *Gastroenterology*, **107**, 1550–5.
[31] Silva, M., Li, X., Cheinquer, H. *et al.* (1992). HCV associated idiopathic thrombocytopenic purpura (ITP), *Gastroenterology*, **102**, A889.
[32] Pivetti, S., Novarino, A., Merico, F. *et al.* (1996). High prevalence of autoimmune phenomena in hepatitis C virus antibody positive patients with lymphoproliferative and connective tissue disorders, *Br. J. Haematol.*, **95**, 204–11.
[33] Dine, G. and Brahimi, S. (1993). Thrombopenie de l'adult associèe a une sèrologie VHC. *Presse Med*, **22**, 269.
[34] Marson, P., Cavatton, G., De Silvestro, G. *et al.* (1994). Screening pretrasfusionale anti-HCV mediante un kit EIA a peptidi sintetici: un confronto con due kit EIA ad antigeni ricombinanti, *La Trasfusione del Sangue*, **39**, 437–43.
[35] Bellentani, S., Tiribelli, C., Saccoccio, G. *et al.* (1994). Prevalence of chronic liver disease in the general population of Northern Italy: the Dyonisos study, *Hepatology*, **20**, 1442–9.
[36] Marson, P., Vicarioto, M., Cavasin, F., Volante, D. and De Silvestro, G. (1991). Prevalenza degli anticorpi anti virus C dell'epatite nell'artrite reumatoide. Studio mediante i test di II generazione. *Rec. Prog. Med.*, **82**, 879–81.
[37] Marson, P., Ostuni, P. A., Vicarioto, M., Ongaro, G. and Gambari, P. F. (1991). Anti-hepatitis C virus serology in primary Sjogren's syndrome: no evidence of cross-reactivity between rheumatoid factor and specific viral proteins, *Clin. Exp. Rheumatol.*, **9**, 661–2.
[38] Luppi, M. and Torelli, G. (1996). The new lymphotropic herpes viruses (HHV-6, HHV-7, HHV-8) and hepatitis C virus (HCV) in human lymphoproliferative diseases: an overview, *Haematologica*, **81**, 265–81.
[39] Albano, A., Pianetti, A., Biffi, M. E. *et al.* (1992). Prevalence of anti-HCV in subjects of various age groups, *Eur. J. Epidemiol.*, **8**, 309–11.
[40] McHutchison, J. G., Wilkes, L. B., Pockros, P. J. *et al.* (1993). Pulse corticosteroid therapy increases viremia (HCV-RNA) in patients with chronic HCV infection, *Hepatology*, **18**, 87A.
[41] Misiani, R., Bellavita, P., Fenili, D., Vicari, O., Marchesi, D., Sironi, P. L. *et al.* (1994). Interferon alpha-2a therapy in cryoglobulinemia associated with hepatitis C virus, *N. Engl. J. Med.*, **330**, 751–6.
[42] Fabris, F., Sgarabotto, D., Zanon, E., Francavilla, F., Zaggia, F., Cadrobbi, P. and Girolami, A. (1993). The effect of a signgle course of alpha-2b-interferon in patients with HIV-related and chronic idiopathic immune thrombocytopenia, *Autoimmunity*, **14**, 174–9.
[43] Durand, J. M., Cretel, E., Retournaz, F., Lefevre, P., Kaplanski, G. and Soubeyrand, J. (1994). Alpha interferon therapy in thrombocytopenia associated with hepatitis C virus infection, *J. Hepatol.*, **21**, 277–9.