# Acquired inhibitors of clotting factors: AICE recommendations for diagnosis and management

Massimo Franchini[1], Giancarlo Castaman[2], Antonio Coppola[3], Cristina Santoro[4], Ezio Zanon[5], Giovanni Di Minno[3,6], Massimo Morfini[7], Elena Santagostino[8], Angiola Rocino[9], on behalf of the AICE Working Group*

*[1]Department of Transfusion Medicine and Haematology, Carlo Poma Hospital, Mantua; [2]Centre for Bleeding Disorders, Careggi University Hospital, Florence; [3]Regional Reference Centre for Coagulation Disorders, Federico II University Hospital, Naples; [4]Haematology, Department of Cellular Biotechnology and Haematology, Sapienza University of Rome, Rome; [5]Haemophilia Centre, Department of Cardiologic, Thoracic and Vascular Sciences, University of Padua Medical School, Padua; [6]President of AICE; [7]Past President of AICE; [8]Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, IRCCS Ca' Granda Foundation, Maggiore Hospital Policlinico, Milan; [9]Haemophilia and Thrombosis Centre, San Giovanni Bosco Hospital, Naples, Italy*
**Co-Authors are listed in the Appendix.*

## Introduction

Autoantibodies affecting the activity or accelerating the clearance of clotting factors (acquired inhibitors) can develop in patients with autoimmune or malignant disorders, but also in subjects without apparent underlying conditions. Such inhibitors are most frequently directed against factor VIII (FVIII) or von Willebrand factor (VWF), giving rise to acquired haemophilia A (AHA) or acquired von Willebrand's syndrome (AVWS), respectively[1,2]. Rarely autoantibody inhibitors can impair the activity of other clotting factors. Indeed, there are descriptions of acquired inhibitors of fibrinogen (FI), factors II (FII), V (FV), VII (FVII), IX (FIX), X (FX), XI (FXI), XII (FXII) and XIII (FXIII), either in children or in adults, with or without associated disorders[3].

Similarly to alloantibodies that occur following replacement therapy in patients with congenital bleeding disorders, acquired inhibitors are type IgG4 polyclonal immunoglobulins (rarely IgM or IgA) targeting several functional epitopes of the clotting factors[4-6]. Their kinetics of inactivation are usually complex (second order or exponential) with rapid initial inactivation, followed by a slower phase or a period of equilibrium in which the factor activity can still be measured[7]. More rarely, these immunoglobulins show first order kinetics, i.e. linear, since the amount of factor inactivated is directly proportional to the concentration of the antibody or to the duration of the incubation. Patients can have a sudden onset of bleeding and, not infrequently, firstly are admitted to hospital wards where clinicians are not experts in the management of coagulation disorders. Rarely, patients do not have bleeding symptoms initially and the diagnosis may be delayed. The severity of the initial bleeding does not predict the severity of subsequent haemorrhagic manifestations[8]. As a consequence, patients with acquired inhibitors to one of the key haemostatic factors are at a high risk of unpredictable severe, sometimes fatal, bleeding, particularly if a definitive diagnosis has still not been made. On rare occasions acquired inhibitors can develop during the puerperium[9-12] or in patients on anticoagulant or anti-platelet treatment[13-16]. In such cases, it is possible that the bleeding is attributed to obstetric or pharmacological reasons. These features do, therefore, make it difficult to recognise the disease and reach the correct diagnosis.

Ideally patients with a deficiency of any clotting factor should be managed in specialised centres for the treatment of haemophilia and inherited bleeding disorders. Indeed, this is recommended by the World Federation of Hemophilia (WFH), numerous experts and national and international guidelines[17-22]. In Italy, patients with acquired inhibitors are mainly managed in Haemophilia Centres (52 centres distributed throughout the country), whose activities are coordinated by the Italian Association of Haemophilia Centres (AICE, *Associazione Italiana Centri Emofilia*). This association promotes an uniform approach to the treatment of patients with congenital bleeding disorders and acquired bleeding syndromes due to autoantibodies. In the 10 years since the publication of the preceding AICE guidelines on the diagnosis and treatment of patients with haemophilia and inhibitors[21], a considerable amount of new information has been published which can be useful for guiding therapeutic choices in patients with AHA, even if no data from prospective, controlled clinical trials are available yet. In contrast, the rarity of inhibitors against other clotting factors strongly influences the possibility of establishing homogeneous treatment practices, based on clearcut evidence. The treatment strategies for patients with such inhibitors are, therefore, largely derived from

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those adopted for patients with AHA. For this reason it is even more important that the therapeutic approach to these very rare haemostatic disorders is as homogeneous as possible, shared among clinicians, and based on available published information, although limited. AICE, therefore, considered it appropriate to revise the previous recommendations on the management of AHA and the general principles of the diagnosis and treatment of inhibitors against other clotting factors. To this end, a specifically convened working group conducted a review of the literature with the primary objective of determining the quality of the evidence available. PubMed was searched using the following keywords: inhibitor, autoantibodies, acquired, h(a)emophilia, von Willebrand disease, factor VIII, IX, II, V, VII, X, XI, XII, XIII, von Willebrand factor, bleeding, management, treatment, factor concentrate, desmopressin, rFVIIa, APCC, immunosuppression, steroid, cyclophosphamide, cyclosporin, immunoglobulins, rituximab, immunoadsorption, immune tolerance induction. The GRADE system was used to define the levels of evidence and the strength of the recommendations derived from the evidence available[23]. The treatment principles and recommendations were collected in a provisional version of this document and submitted to the consideration of AICE members and, finally, approved during the AICE General Assembly on November 3rd, 2014.

## Cumulative incidence

AHA is a rare disease, with an incidence in the general population of about 1.5 case per million persons/year[24-26]. Its frequency increases with age, since the condition is extremely rare in children[27-29] and becomes significantly more common in patients over the age of 65 years[8]. The median age at diagnosis was 78 and 74 years in the two largest cases series so far published, a prospective study carried out in the United Kingdom[8] and the European Acquired Haemophilia (EACH2) registry[29], in which more than 80% of the patients were 65 years old or over. The incidence appears to be similar in males and females, except in the age group from 20 to 40 years, because of the pregnancy-associated cases[9-12]. This rare condition was observed in about 1:350,000 deliveries in the study carried out in the United Kingdom[8] and in 20 cases during 15 years of observation in a study carried out in Italy by AICE[30]. More recently, an analysis of the EACH2 registry revealed 42 cases of post-partum AHA, eight of which were, however, already detectable prior to delivery[12].

The incidence of acquired deficiency (AD) of other clotting factors: fibrinogen (FI, AFID), FII (AFIID), FV (AFVD), FVII (AFVIID), FIX (acquired haemophilia B, AHB), FX (AFXD), FXI (AFXID), FXII (AFXIID) and FXIII (AFXIIID) is much less known, since only sporadic cases have been described, usually associated with autoimmune disorders and neoplastic conditions[3]. Acquired forms of VWD are rare and clinically similar to the congenital disease. They are characterised by prolonged bleeding time and variable reductions in the plasma levels of FVIII and VWF[31]. The pathophysiology of these conditions is more complex, because various mechanisms can cause acquired alterations to VWF (Table I).

The diagnosis of these disorders may be problematic and a negative personal and family history for bleeding symptoms is an important clue to the diagnosis. These patients often present with an electrophoretic pattern similar to that of the type 2 congenital disease, with variable reductions in the higher molecular weight VWF multimers[32-34]. Furthermore, it should be underlined that heterogeneous acquired alterations in the multimeric structure of VWF are relatively common in a number of conditions, but these are often not associated with a definite bleeding risk. Bleeding, on the other hand, often occurs in lymphoproliferative disorders (frequently monoclonal gammopathies) in which the presence of antibodies, able to complex with VWF and causing its accelerated clearance from the circulation, can be demonstrated, even indirectly. These forms are most frequently associated with a significant reduction of the circulating levels of VWF and are specifically discussed in this document. So far, 186 cases of AVWS from 50

**Table I** - Pathophysiological mechanisms of acquired abnormalities of von Willebrand factor and associated conditions.

| Pathogenic mechanism | Associated conditions |
|---|---|
| 1) Specific or non-specific autoantibodies generating immune complexes with vWF, increasing its clearance from the circulation | - Lymphoproliferative disorders<br>- Tumours<br>- Immunological disorders |
| 2) vWF adsorption onto cell membranes of tumour cells or other surfaces | - Lymphoproliferative disorders<br>- Wilms' tumour<br>- Myeloproliferative disorders |
| 3) vWF degradation by increased shear stress | - Congenital heart disease<br>- Aortic stenosis (Heyde's syndrome)<br>- Endocarditis<br>- Severe atherosclerosis<br>- Beta thalassaemia major |
| 4) Reduced vWF synthesis | - Hypothyroidism |
| 5) Increased vWF proteolysis by specific proteases | - Myeloproliferative disorders<br>- Uraemia<br>- Ciprofloxacin |
| 6) Increased vWF proteolysis by non-specific proteases | - Primary hyperfibrinolysis<br>- Secondary hyperfibrinolysis<br>- Fibrinolytic therapy |
| 7) Idiopathic | - Valproic acid<br>- Amyloidosis<br>- Viral infections<br>- Mixed cryoglobulinaemia |

Centres throughout the world have been entered in the International Registry of the syndrome, held by the von Willebrand Factor Subcommittee of the International Society on Thrombosis and Haemostasis. These patients are divided equally into males and females and have a mean age of about 56 years[34]. There are currently no prospective studies indicating the real incidence of this rare acquired disorder[35].

## Risk factors

In about half of the cases of AHA, inhibitor onset occurs in the presence of disorders or clinical conditions more likely to be associated with development of autoantibodies. In particular, most frequently solid or haematological neoplasms and autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus), but also pregnancy (particularly the puerperium), the use of some drugs (especially antibiotics and interferon), and dermatological diseases have been reported in about 50% of cases (Table II). In the other half of the cases no disorders or triggering conditions are detectable and these cases are referred to as idiopathic[2,8,28,29,36-39]. It is not uncommon that the diagnosis of AHA precedes that of the associated condition and triggers the investigations leading to the diagnosis of the latter[2,37]. Table II shows the distribution of idiopathic cases and of the different conditions associated with "secondary" AHA in the largest case series published in the literature.

Inhibitors against FV are the most frequent among the inhibitors against the other clotting factors. In most cases these inhibitors develop in association with a readily identifiable risk factor, such as a surgical intervention, antibiotics (particularly beta lactams and aminoglycosides), blood transfusions, malignancies and autoimmune diseases[40]. Historically, cases of AFVD were described following the use of topical haemostatic agents containing bovine thrombin and traces of bovine FV able to stimulate an immune response also cross-reacting with human FV[41-42]. The replacement of this product with recombinant human thrombin has led to a considerable decrease in the incidence of AFVD[43].

Like anti-FVIII autoantibodies, acquired inhibitors of FIX (AHB) frequently develop in association with autoimmune diseases[44,45], although post-partum cases have also been described[46].

Inhibitors against FVII, FX and FXI have been reported occasionally, mostly in association with autoimmune diseases[47-53]. In a recent review, Lee *et al*. described 34 cases of anti-FX inhibitors not related to amyloidosis, a quarter of which were associated with malignancies, including four cases of acute myeloid leukaemia, while in 38% of the cases a respiratory tract infection was present[54]. Anti-FVII inhibitors have been reported following the use of various drugs, including some antibiotics[55]. Finally, anti-FXIII inhibitors can prevent activation of FXIII by thrombin or prevent its binding to specific sites of fibrin[56]. The clinical picture may be more severe than that of patients with a congenital deficiency. Common manifestations are haematomas, haemarthroses, intracranial bleeding, post-operative haemorrhage and delayed wound healing. Recurrent abortions may be the presenting manifestation in a few women[56].

Starting from the first description in 1968 of a case of AVWS associated with systemic lupus erythematosus[57], the International Registry and other Authors have found that 47% of the cases are associated with lymphoproliferative and 19% with myeloproliferative disorders respectively, in addition to 13% of cases associated with cardiovascular diseases and 7% with solid tumours. Post-partum cases have been described much more rarely. Thus, overall, the conditions most frequently associated with AVWS are lympho-myeloproliferative diseases which, alone,

**Table II -** Prevalence of idiopathic and secondary acquired haemophilia A reported in studies including more than 60 patients.

| | Green & Lechner 1981[39] (n=215) | Morrison 1993[36] (n=68) | Delgado 2003[37] (n=234) | Collins 2007[8] (UKHCDO; n=172) | Knoebl 2012[29] (EACH2; n=501) | Borg 2013[28] (SACHA; n=82) |
|---|---|---|---|---|---|---|
| Idiopathic | 44% | 55% | 58% | 53% | 52% | 55% |
| Solid or haematological tumours | 6% | 12% | 18% | 15% | 12% | 22% |
| Autoimmune diseases | 17% | 12% | 9% | 17% | 13% | 15% |
| Pregnancy | 7% | 11% | 15% | 2% | 8% | 7% |
| Drugs | 5% | 3% | nr | nr | 3% | nr |
| Dermatological disorders | 4% | 2% | nr | 3% | 1% | nr |
| Other conditions | 17% | nr | nr | nr | 16% | 1% |

UKHCDO: United Kingdom Haemophilia Centre Directors Organisation; EACH2: European Acquired Haemophilia Registry; SACHA: *Surveillance des Auto antiCorps au cours de l'Hémophilie Acquise* (prospective French registry); nr: not reported.

account for 48-63% of cases[58-63]. However, in the recently established German Registry, AVWS is more frequently associated with congenital or acquired heart diseases, even though the clinical impact is often uncertain[33].

## Diagnosis

An anti-FVIII inhibitor or, exceptionally, an acquired anti-FIX inhibitor should be suspected in the presence of unexpected bleeding, often severe, which occurs spontaneously or after minor trauma, following invasive procedures (e.g. placement of a venous catheter, endoscopic investigations, arterial blood sampling, intramuscular injections) or surgical interventions in patients without a personal or family history of bleeding[64]. Frequent sites of bleeding are the skin (vast ecchymoses and subcutaneous haematomas), mucosae (epistaxis, bleeding gums, metrorrhagia, gastrointestinal bleeding), and muscles[2,8,29,65]. Unlike in congenital haemophilia A and B, haemarthroses are rare[2,64,65]. Retroperitoneal bleeding occurs in 20% of cases and can be fatal. Bleeding in critical sites (larynx, nerves, vessels) can give rise to symptoms due to compression. The bleeding manifestations of patients with acquired inhibitors are generally more severe than those of patients with congenital haemophilia with or without inhibitors[38]. Severe bleeding, including those in the gastrointestinal tract and retroperitoneal space, occurs in up to 70-90% of cases. The severity of bleeding, together with delays in diagnosis, the advanced age of patients, associated disorders and inadequate management are all concurrent causes of the reported high rate of mortality (up to 41%) associated with this acquired bleeding disorder[2].

The key diagnostic laboratory picture for the diagnosis of AHA is a prolonged activated partial thromboplastin time (APTT), not corrected by normal plasma (mixing test), with a normal prothrombin time (PT)[65]. Faced with a clinical suspicion of inhibitors and the finding of a prolonged APTT, the mixing test must be carried out quickly, even as an emergency laboratory test. The APTT of mixtures of the patient's plasma and normal plasma in different proportions (volume/volume ratios 1:4, 1:1 and 4:1) must be determined before and after incubation at 37 °C for at least 2 hours, because the inactivation of FVIII by autoantibodies is time- and temperature-dependent. Therefore, the APTT of the mixture is abnormally prolonged after incubation, whereas it can be corrected immediately after mixing. However, given the short incubation time, this method may not detect inhibitors with complex or "slow", time-dependent kinetics, or very low titre inhibitors, present in amounts below the sensitivity limit of the method. Furthermore, the presence of heparin and lupus anticoagulant (LA) inhibitors must be excluded. The presence of heparin is suggested by a prolonged thrombin time (TT) with a normal reptilase time[66]. Prolonged APTT values of the mixture of patient's plasma with normal plasma which are similar at time 0 and after incubation are indicative of LA, except in cases in which the LA also has anti-FVIII activity. The presence of LA can then be confirmed by specific tests, such as the diluted Russell viper venom time (dRVVT) and tests at low phospholipid concentrations, e.g. the kaolin clotting time (KCT) and silica clotting time (SCT), followed by confirmatory tests at high phospholipid concentrations, such as the platelet neutralisation procedure (PNP) or hexagonal phase phospholipid test[65,67]. The diagnosis of inhibitor is then confirmed by a specific assay of the factor and titration of the inhibitor by the Nijmegen modification of the Bethesda method[68]. Sometimes an anti-FVIII inhibitor, particularly if at high titre, can interfere non-specifically with assays of other factors of the intrinsic pathway, leading to the finding of falsely reduced activity of FIX, FXI and FXII; in these cases, diluting the sample being tested with buffer solution reduces this interference, leading to normalisation of the activity of the other factors except FVIII, which continues to be inhibited even at lower concentration by the specific autoantibody[69]. Finally, it should be considered that rare circulating inhibitors that interfere with the activity of several clotting factors have been reported, particularly in patients with cancer. These inhibitors are not autoantibodies, but endogenous glycosaminoglycans with anticoagulant activity, the so-called "heparin-like" inhibitors[70-72]. Figure 1 is an algorithm for the diagnosis of AHA.

As far as AVWS due to autoantibodies is specifically concerned, the presence of inhibitors is documented by evaluation of VWF activity after incubating the plasma sample for 2 hours at 37 °C with normal plasma (mixing test at different concentrations)[34]. It should be noted that results of this test may be difficult to be interpreted. For this reason it may be necessary to evaluate the type and duration of response to desmopressin (DDAVP) and/or FVIII/VWF concentrate in subjects with a suspected AVWS, in order to determine whether these patients have an accelerated clearance of the FVIII/VWF complex, which would confirm the suspect of a specific antibody.

A prolonged APTT, with normal PT, is the key laboratory finding also for the diagnosis of the very rare cases of AHB and AFXID. Prolongation of both the APTT and PT, not corrected by a mixing test with normal plasma, is indicative of the presence of an AFIID, AFVD, or AFXD. In the case of an AFIID, the TT is notably prolonged, while the reptilase time is normal. In contrast, the TT is normal in the presence of an anti-FX inhibitor. Prolongation of the PT which

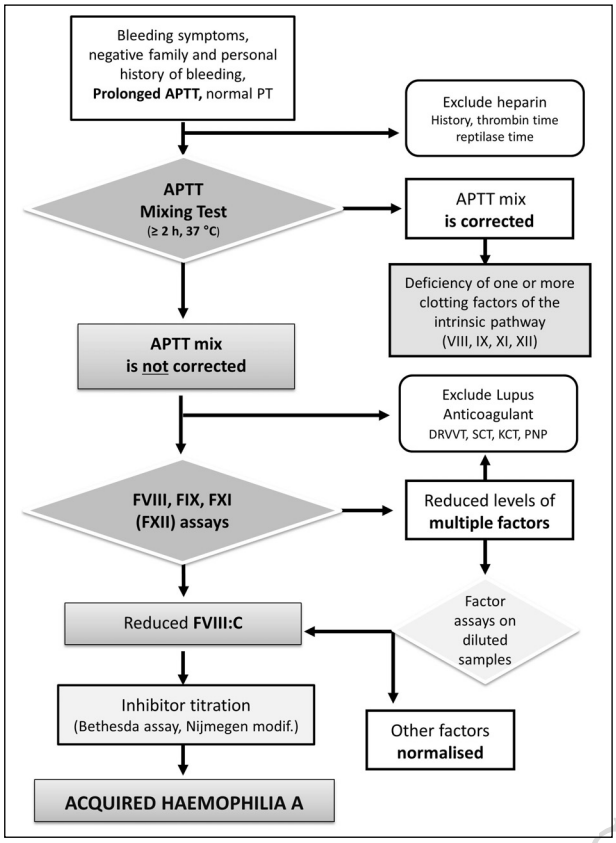


**Figure 1 -** Algorithm for the laboratory diagnosis of acquired haemophilia A.
See the text for details. An APTT (activated partial thromboplastin time) not corrected at mixing tests is also found in the presence of lupus anticoagulant (see the text for details for differential diagnosis). However, this condition is not usually associated with bleeding manifestations. Similarly, abnormalities of factor XII does not result in a bleeding tendency. PT: prothrombin time; DRVVT: diluted Russell viper venom time; SCT: silica clotting time; KCT: kaolin clotting time; PNP: platelet neutralisation procedure; FVIII:C: factor VIII coagulation activity.

fail to correct by the addition of normal plasma, together with a normal APTT, is the cornerstone of the laboratory diagnosis of AFVIID. Figure 2 summarises the laboratory approach to the diagnosis of acquired inhibitors to clotting factors on the basis of screening tests, taking into consideration some clinical conditions, particularly iatrogenic ones, that cause similar alterations in these tests. It should be emphasised that in the case of AFXIIID the coagulation screening tests are normal. The only diagnostic laboratory test for this condition is the FXIII assay, measuring the patient's sample and its mixing with normal plasma. By contrast, in the presence of a FI inhibitor, the PT, APTT, TT and reptilase time are all prolonged and plasma fibrinogen level, assayed with the Clauss functional method, is reduced. In the mixing test, the TT is not corrected by the addition of normal plasma. In all cases, a modified Bethesda test can confirm the presence of the inhibitor and provide its level.

Given the particular features of the methods used, the laboratory diagnosis of patients with suspected acquired inhibitors against a clotting factor must be made/confirmed in specialised coagulation laboratories that work in close collaboration with centres with expertise in the diagnosis and management of patients with congenital haemophilia and other inherited bleeding disorders.

## Treatment

The presence of autoantibodies against clotting factors induces a high risk of bleeding which requires immediate treatment aimed at eradicating the inhibitor[73]. The search for any associated conditions is equally important because, in some cases, the treatment of such conditions can lead to the disappearance of the inhibitors and modify the prognosis[2,37,74,75]. A thorough history including information on acute or chronic assumption of drugs and any recent delivery (or termination of pregnancy) is a prerequisite for a correct diagnosis and eradication of the inhibitor. Nevertheless, it should be remembered that in about 50% of cases no underlying condition is identifiable. If bleeding is present, immediate evaluation and appropriate therapy is urgently required. For this reason it is strongly recommended that patients with acquired inhibitors of clotting factors are managed in specialised centres for the diagnosis and treatment of haemophiliacs with inhibitors. Thanks to national and international registries established in the last decade, much information on the treatment of AHA has been obtained and has enabled international recommendations to be drawn up by a group of experts[22]. Likewise, the International Registry of AVWS is an important instrument for collecting data on this condition[33-35]. The information on management of acquired inhibitors against other clotting factors remains much more limited as a consequence of their rarity.

### Treatment and prevention of bleeding

Bypassing agents (recombinant activated factor VII [rFVIIa] and activated prothrombin complex concentrate [APCC]) are the first-line treatments of bleeding episodes in patients with AHA[2,64,76,77]. Both types of bypassing agents have been demonstrated to be effective and no comparative studies able to demonstrate the superiority of one product over the other are available[64]. It should be noted that the treatment approaches with these agents (doses and administration regimens) are based wholly on the experience of their use in congenital haemophiliacs with inhibitors, despite the considerable difference in the type of patients with acquired inhibitors

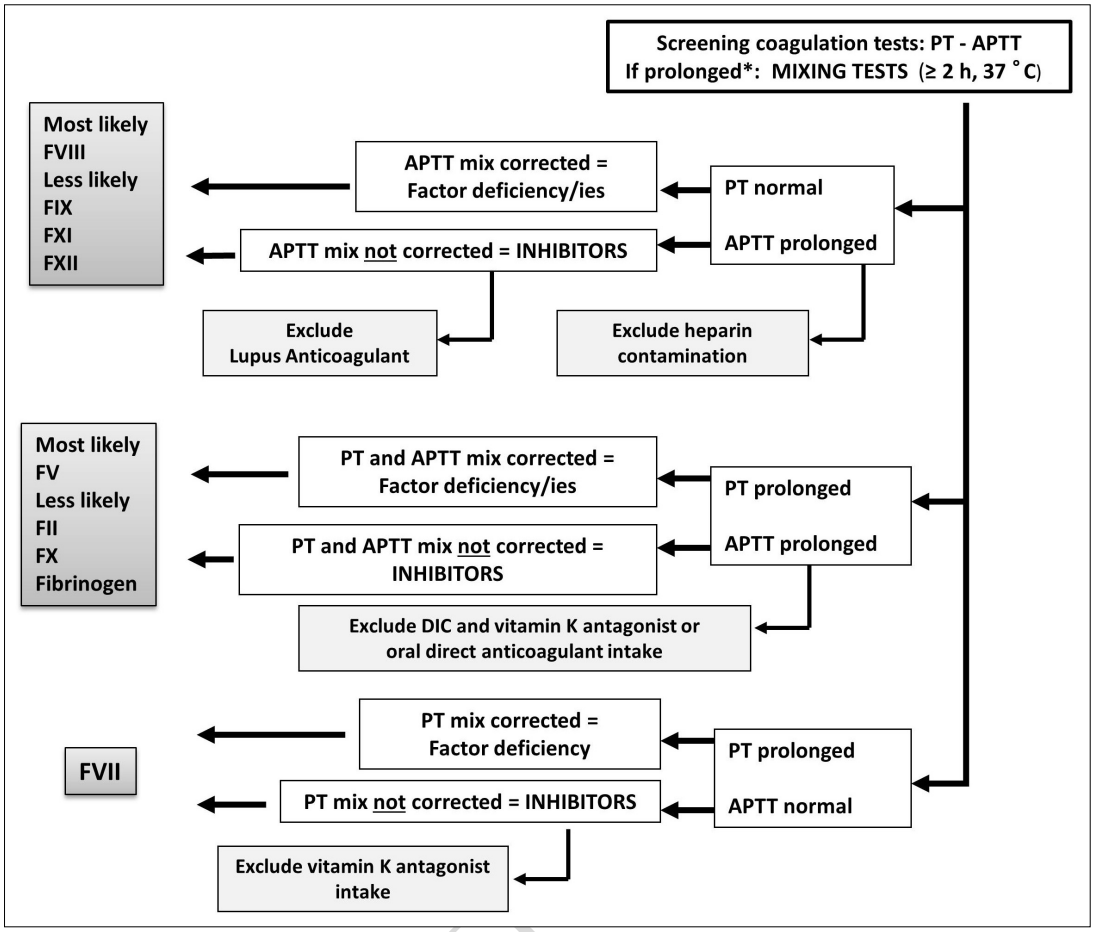


**Figure 2 -** Identification of abnormalities of clotting factors, including acquired inhibitors, from the evaluation of screening coagulation tests.
* In the case of inhibitors of fibrinogen and FII, thrombin time is also prolonged and not corrected at mixing tests. Screening tests are normal in the presence of inhibitors against FXIII. The specific FXIII assay with measurements on the patient sample and on mixture with normal plasma is needed for identifying inhibitors.
PT: prothrombin time; APTT: activated partial thromboplastin time; FVIII: factor VIII; FIX: factor IX; FX: factor X; FXI: factor XI; FV: factor V; FII: factor II; FX: factor X; FVII: factor VII.

(often elderly, and/or with comorbidities) and of the bleeding events (mainly muco-cutaneous, haemarthroses rare). APCC (FEIBA®, Baxter, Deerfield, IL, USA) is a plasma-derived product whose efficacy in AHA has been documented by various reports in the literature and by a retrospective review of 34 patients, which showed an overall efficacy of 86% when the treatment was used as first-line at a typical dose of 75 U/kg every 8-12 hours[78]. The median number of infusions necessary to control a severe bleeding episode was ten, while six infusions were sufficient in the case of moderate bleeding. Recombinant FVIIa (NovoSeven®, NovoNordisk, Bagsvaerd, Denmark) has the advantage of being free of the risk of transmission of blood-borne pathogens. Its efficacy in AHA has been documented by the experience in 139 patients in whom it was used as first-line treatment, with effective control of bleeding in 95%[79]. The median dose administered was 90 µg/kg, although there was considerable variability in the dose (60-160 µg/kg), number of infusions (1-33) and duration of treatment (1-7 days). In the EACH2 Registry, rFVIIa was the most frequently used haemostatic agent, with a success rate of controlling bleeding (92%) similar to that of APCC (93%)[77]. The main concern related to the use of these bypassing agents remains the risk of thrombosis with which they are associated[80,81]. The EACH2 Registry reports 11 thrombotic adverse events (7 arterial and 4 venous), which is an incidence higher than that reported in patients with congenital haemophilia and inhibitors[77]. There are no significant differences between the two bypassing agents with regards to this complication and two cases were reported in patients who did not receive any haemostatic treatment. Thus, the higher incidence of thrombotic events in patients with acquired inhibitors is probably due to the older age of these patients and the greater frequency of concomitant

cardiovascular and thrombotic risk factors[77]. The initial dose of rFVIIa should be 90-120 μg/kg, followed by additional infusions 2-3 hours apart, while that of APCC should be 50-100 U/kg every 8-12 hours, taking care not to exceed the maximum daily dose of 200 U/kg. Higher doses of either agent are not indicated because of the increased risk of thrombosis[19]. In the case of clear inefficacy of one or other of the agents, a switch to the alternative agent should be considered and the change should be performed early, in order to prevent persistent bleeding leading to disabling sequelae or life-threatening situations[19,82].

Alternative treatments, aimed at increasing the levels of circulating FVIII, include DDAVP and FVIII concentrates. DDAVP can be used in patients with anti-FVIII autoantibodies and measurable FVIII levels, without inducing an anamnestic response[83]. The therapeutic efficacy is, however, unpredictable[83]. Further limitations of this treatment are the development of tachyphylaxis (loss of response after consecutive injections of several doses) and the risk of fluid retention. In patients with a low-titre inhibitor (<5 BU/mL), replacement treatment with FVIII can be considered at doses able to neutralise the inhibitor and increase the amount of circulating FVIII[21,84,85]. It should, however, be noted that calculations by means of the usually adopted formula for determining the neutralising dose are strongly limited by the inaccuracy of laboratory methods for determining the inhibitor titre[21]. The level of FVIII should, therefore, be controlled 15 to 30 minutes after completion of the FVIII concentrate infusion. Furthermore, the likelihood of therapeutic failure, with lack of a haemostatic response, observed in the EACH2 study, was markedly higher in the case of the use of FVIII or DDAVP, compared to bypassing agents treatment (31.4% *vs* 8.2%, p<0.001)[77]. However, FVIII concentrates have recently been used successfully in some patients in the elderly and/or at high cardiovascular risk[86,87]. The number of patients was, however, limited and further experience in these settings is needed to confirm the results. If this therapeutic option is chosen, careful clinical and laboratory follow up is indicated, with levels of FVIII monitored at least daily. Such monitoring has a dual purpose: checking that the doses of FVIII concentrate are haemostatically effective and early detecting of development of an anamnestic response. In the latter case, it is crucial to evaluate whether adjustment of the doses of FVIII would enable continued neutralisation of the inhibitor. No thromboembolic events have been reported in patients treated with FVIII concentrates[77].

High doses of FVIII (100 IU/kg) can be given after removing the inhibitor by immunoadsorption, which is achieved by filtering the patient's plasma through a sepharose column with bound recombinant protein A (Immunosorba®, Excorim AB, Lund, Sweden), thus resulting in levels of circulating FVIII sufficient to ensure adequate haemostasis, even in patients with high levels of inhibitors[88]. This strategy may be useful in patients who require elective surgery or if bypassing agents are ineffective, although immunoadsorption technology is only available in a limited number of centres[89].

FVIII concentrates derived from porcine plasma were, in the past, used successfully in patients with low cross-reactivity[36,90], but are no longer available. Very recently, however, the American Food and Drug Administration (FDA) approved the marketing of a recombinant B-domain deleted factor VIII of porcine origin, OBI-1, with the indication for use in patients with AHA. At initial dose of 200 U/kg and subsequent adjustments to maintain FVIII target levels, irrespective of inhibitor titre and anti-porcine FVIII cross-reactivity, OBI-1 was effective in the control of bleeding episodes in 86% of cases[91]. This product is under evaluation by the European Medicines Agency (EMA) for the same indication.

The following therapeutic approaches can be used, in sequence, with the aim of preventing and/or managing bleeding in patients with AVWS: DDAVP, FVIII/VWF concentrates, high-dose immunoglobulins (HDIg), and rFVIIa at doses similar to those recommended for patients with AHA. In particular, HDIg (0.4 g/kg/die for 5 days or 1 g/kg/die for 2 days) have been used with success in cases of AVWS associated with IgG monoclonal gammopathy of undetermined significance[92], whereas their efficacy in IgM gammopathies is less clear. Plasmapheresis and immunosuppressive treatment may be useful in cases of severe bleeding.

The treatment of bleeding episodes in patients with acquired inhibitors against other clotting factors depends largely on the specificity and the level of the inhibitor. In some cases the antibody can be neutralised by using high doses of purified concentrates of the deficient factor. This is the case of patients with acquired inhibitors to FI, FVII, FIX, FXI, and FXIII, especially if they are at a low titre. However, the factor level actually reached must be monitored closely in order to adjust the doses and administration intervals and to ensure that haemostatically effective levels are being reached. APCC may be specifically indicated as the treatment of choice in patients with AFVIID and high inhibitor level and in patients with AFIID and AFXD, especially in the presence of a low antibody titre. An alternative to APCC for patients with AFIID and AFXD could be the use of a prothrombin complex concentrate (PCC). Meticulous monitoring of the specific factor level reached is, however, indicated if this product is used. It should

also be kept in mind that repeated infusions of PCC and APCC can lead to an accumulation of other clotting factors, thus increasing the risk of thromboembolic events. Furthermore, the use of fresh-frozen plasma is not indicated in most cases because it is usually impractical to infuse volumes sufficient to provide factor concentrations able to neutralise the inhibitor and achieve haemostatic levels of the deficient factor, without the risk of circulatory overload. Nevertheless, the use of this blood product and platelet concentrates is the main therapeutic option for patients with AFVD. APCC can be considered as an alternative to fresh-frozen plasma in these patients, particularly if they have a high risk of cardiovascular complications.

Finally, with regards to the use of PCC and fresh-frozen plasma, AICE recommends the use of solvent/detergent (S/D) virally-inactivated products manufactured from national plasma collected from voluntary and habitual unpaid donors throughout the network of Italian Transfusion Services. If not available, preparations of commercial S/D virally-inactivated products must be preferred. Furthermore, the use of plasma preparations virally inactivated by in-house methods is not recommended, because clotting factor concentrations cannot be standardised in these products, at least on the basis of current legislation[93], that does not call for assays of the single clotting factors in individual units of plasma collected.

Tranexamic acid may be a useful treatment in the case of non-severe mucosal bleeding or in association with other haemostatic agents, again, in particular in patients with mucosal bleeding. The only contraindication is the presence of renal tract bleeding[94]. Administration in association with activated clotting factor concentrates, in particular with APCC, however, should be considered cautiously, taking into account the age of the patients and the presence of comorbidities which are known to be associated with a higher risk of thrombosis.

**Inhibitor eradication**

Before considering any eradication therapy, the removal or successful management of conditions which could have triggered the development of the autoantibody (e.g. cancer, drugs, autoimmune diseases), if possible, is a priority and this could lead to rapid disappearance of the inhibitor. Inhibitor eradication is based on immunosuppressive therapy[2,22,64]. This approach is aimed at inhibiting or eliminating the cellular clone responsible for synthesising the autoantibodies. The drugs most frequently used are prednisone, cyclophosphamide, azathioprine, vincristine, cyclosporine, HDIg and anti-CD20 monoclonal antibody (rituximab), administered as single therapies or in various combinations[2,3]. Prospective, controlled clinical trials to evaluate the efficacy of the different treatments are not available, partly because of the possible spontaneous remissions (paediatric cases, pregnancy- and drug-related cases). Prognostic factors predicting success of eradication therapy are a low-titre inhibitor and a short period between appearance of the inhibitor and the start of immunosuppressive therapy[95]. In 30% of cases, however, the autoantibody may disappear spontaneously[96], a phenomenon that is not predictable but is common if the inhibitor is pregnancy- or drug-related. Different strategies can, therefore, be adopted in different types and subgroups of patients[37]. A "watch and wait" approach may be indicated in children, in cases associated with pregnancy and the use of drugs; combined immunosuppressive therapy is usually started in idiopathic cases or in those associated with cancer or autoimmune diseases, given the risk of life-threatening bleeding if the antibody persists. Steroids (prednisone 1-2 mg/kg/die for 4-6 weeks) as monotherapy or in association with cyclophosphamide (1-2 mg/kg/die for a maximum of 5 weeks)[18-22,97-104] are the most commonly used first-line therapeutic strategies, with 70-80% of cases of successful inhibitor eradication in AHA. The only prospective, randomised study on this issue was performed early in 1993 and involved 31 patients treated with prednisone monotherapy at a dose of 1 mg/kg/die for 3 weeks[102]. If the inhibitor was not eradicated, the patients were randomised to receive the same dose of prednisone or prednisone + cyclophosphamide (2 mg/kg/die) or cyclophosphamide monotherapy, all for 6 weeks. About one-third of the patients successfully responded to the initial treatment with the steroid, while about 50% of the resistant patients also failed to respond to the subsequent treatment including cyclophosphamide. No difference was seen in the rates of inhibitor eradication between the patients in the different treatment arms. The results of a prospective, non-randomised study carried out in the United Kingdom showed similar percentages of inhibitor eradication[8] among 34 patients treated with steroid monotherapy and 45 patients treated with steroids in combination with cytotoxic agents (76% *vs* 78%, respectively). Furthermore, the mortality rate in the two groups was not statistically different. In a meta-analysis conducted by Delgado *et al*. on 20 studies, cyclophosphamide was found to be superior to prednisone in terms of eradication of the inhibitor, but was not associated with an improvement of overall survival[37]. This finding was attributed to the greater toxicity of cyclophosphamide and, in particular, to the increased mortality caused by infections. A more recent meta-analysis of 32 non-randomised studies showed that, compared to patients treated with steroid monotherapy, patients receiving combined schedules

had a lower probability of persistent inhibitor and a lower risk of mortality[103]. In the recent EACH2 Registry the combination of steroids and cyclophosphamide was associated with a higher percentage of inhibitor eradication compared to treatment with steroids alone (80% *vs* 58%; 70% *vs* 48% considering relapses), although again there were no differences in survival of the patients[104]. In conclusion, the data currently available seem to indicate that, although the association of steroids and cyclophosphamide is more likely to achieve stable eradication of the inhibitor than steroids alone, this advantage does not affect the patients' survival, probably because of the greater toxicity of cyclophosphamide. For this reason, cyclophosphamide and other cytotoxic agents must be used cautiously, especially in elder patients. The approach to patients with pregnancy-related AHA also requires particular care because, as above mentioned, spontaneous remission of the inhibitor is possible[30,96] and both the age of the patient and the possible side effects of cyclophosphamide and other alkylating agents in fertile women must be taken into account. Furthermore, although recurrence of the inhibitor in subsequent pregnancies is rare, this possibility must be considered. No recurrences were reported in the Italian study conducted by AICE[30], although Solymoss reported recurrences in four of six subsequent pregnancies in three women with AHA[9]. Finally, it should not be overlooked that the inhibitor in the mother can influence the level of FVIII in the foetus at the time of delivery[105].

With regards to the use of HDIg, the available data indicate that these, alone or in association with steroids, are not able to increase the rate of inhibitor eradication, whereas they can play an important role in the management of the autoantibodies associated with AVWS[8,37,106].

Interesting results have been reported with the use of cyclosporine, although the experience is still not sufficient to define its real advantages[107]. In contrast, there is ever increasing evidence on the efficacy of anti-CD20 monoclonal antibody (rituximab), in particular in patients with relatively low-titre inhibitors resistant to other treatments[108-110]. In a literature review of 65 patients treated with rituximab in association with various other immunosuppressive agents, the efficacy was greater than 90% although the lack of a control group did not allow possible biases to be excluded[109]. Similar success rates were reported in a study in which 42 patients treated with rituximab were compared to 44 patients treated with steroids+cyclophosphamide[110]. In the EACH2 Registry, 30 of 51 patients (59%) treated with a therapeutic regimen that included rituximab achieved stable inhibitor eradication[104]. However, the efficacy was greater when rituximab was associated with other immunosuppressive agents, resulting in success rate similar to that observed in patients treated with steroids and cyclophosphamide (64% *vs* 70%, respectively). Rituximab has also been shown to be effective in women with post-partum inhibitor[12,111,112], even if the data currently available do not indicate that the monoclonal antibody produced better results than other treatments. Rituximab should, therefore, be used as a second-line treatment in patients not responsive to treatment with steroids or a combination of steroids + cyclophosphamide and in the case of contraindications to the use of other immunosuppressive drugs.

Immune tolerance induction regimens have rarely been used in the treatment of AHA. The efficacy and safety of such an approach do, however, seem to be indicated by the use of the Budapest protocol, in which the administration of human FVIII at the daily dose of 30 UI/kg for the first week, 20 UI/kg for the second week and 15 UI/kg for the third week is combined with intravenous administration of cyclophosphamide at a daily dose of 200 mg (total dose 2-3 g) and systemic methylprednisolone at a daily dose of 100 mg for the first week followed by a gradual tapering in the subsequent 2 weeks[113]. This protocol produced complete, stable remission in more than 90% of the patients treated. Similar results were obtained by the group in Heidelberg using the modified Malmö protocol which involves immunoadsorption of the inhibitor on staphylococcal protein A, together with the administration of high doses of FVIII, cyclophosphamide and corticosteroid[114,115]. The major limitation of the studies on the use of FVIII concentrates in immune tolerance regimens is, however, the lack of a control group. The data available do not, therefore, seem sufficient to determine whether the administration of FVIII does contribute to increasing the efficacy of immunosuppressive therapy.

The occurrence of side effects must always be kept into account during treatment with immunosuppressive drugs. Infections, neutropenia and diabetes mellitus are the most common adverse events, but hypertension, osteoporosis, psychosis, and cataract development following steroid use in elderly patients can also occur. Furthermore, a not negligible rate of death (3-12%) has been reported in recent series, in most cases due to the development of infections during immunosuppressive treatment[8,104].

Successful eradication requires that the inhibitor titre is "negative" (<0.6 BU/mL) and that the levels of FVIII are persistently normal (>70%) once treatment is discontinued[104]. The risk of inhibitor recurrence in the various recent series ranges between 10% and 20%. Recurrence occurred after a median of 7.5 months (range, 1 week - 14 months) in 20% of 102 patients included in the prospective study carried out in the United Kingdom[8]. In the recent EACH2 registry,

inhibitors recurred after a median of 4 months in 18% of the patients treated with steroids alone, in 12% of the patients treated with a combination of steroids and cyclophosphamide and in 1% of the patients treated with rituximab[104]. Prolonged follow up is necessary to determine whether inhibitors have been stably eradicated and, once the FVIII levels have been normalised, monitoring must be continued at least monthly for the first 6 months[22] and subsequently every 3 months or less frequently, depending, in part, on the evolution of any autoimmune or malignant disorders previously associated with the development of the inhibitor. The FVIII levels should also be monitored in subsequent years, in particular if the patient must undergo surgery or other invasive procedures at high bleeding risk.

It is also important to monitor FVIII levels during eradication therapy in order to assess the risk of thromboembolism which could be increased in some patients because of their advanced age, pre-existing comorbidities and immobilisation, in addition to the risk derived from the haemostatic treatment with activated clotting factor concentrates. In such patients, the use of mechanical (e.g. elastic compression stockings) and/or pharmacological prophylactic antithrombotic measures should be considered, particularly when the inhibitor has, presumably, been eradicated and the FVIII levels can be markedly higher than normal, adding as a further risk factor for thromboembolism[2,22,116,117].

There are extremely few data in the literature concerning eradication of inhibitors of other clotting factors. For this reason, treatment with immunosuppressive agents is indicated only in the case of frequent and severe bleeding, particularly because resolution of the associated autoimmune disease or cancer often leads to remission of the inhibitor. However, in the most severe cases, eradication therapy can be attempted, using the same regimens as those widely used in patients with AHA.

## Summary of the recommendations on the diagnosis, treatment and clinical monitoring of patients with acquired inhibitors

On the basis of the evidence available in the literature, the following recommendations concerning the clinical and laboratory criteria for the diagnosis of acquired haemophilia A and acquired inhibitors of other clotting factors can be summarised:

- The diagnosis of AHA must be considered in the case of a recent-onset, unexpected bleeding in a patient without personal and family history of bleeding who exhibits an isolated prolonged APTT that is not corrected by the addition of normal plasma in a 1:1 mixing test and incubation for at least 2 hours at 37 °C **(Grade 1B recommendation)**.
- The presence of acquired inhibitors against functional epitopes of other clotting factors must be considered in the case of a recent-onset, unexpected bleeding in a patient without personal and family history of bleeding who shows a prolonged PT and/or APTT and/or TT that are not correct by the addition of normal plasma, in a 1:1 mixing test and incubation for at least 2 hours at 37 °C **(Grade 1B recommendation)**. Inhibitor against FXIII should be ruled out by specific test if screening tests are normal.
- The laboratory diagnosis of patients with a suspected acquired inhibitor against a clotting factor must be made/confirmed by specialised coagulation laboratories working in close collaboration with centres with expertise in the diagnosis and treatment of congenital haemophilia and other inherited bleeding disorders **(Grade 1B recommendation)**. These laboratories must ensure quick execution of the mixing test with normal plasma and assays of clotting factor levels, even in an emergency context **(Grade 1B recommendation)**.

The treatment of bleeding manifestations in patients with AHA, AVWS and inhibitors of other clotting factors must be guided by taking into account the following recommendations:

- Not all patients manifest clinically relevant bleeding when an acquired inhibitor develops/is diagnosed. In such cases treatment with haemostatic agents may not be required and the patients may be managed conservatively adopting a "wait and watch" approach **(Grade 2C recommendation)**.
- When possible, the removal of the condition that probably triggered the development of the inhibitor (e.g. cancer, drugs) should be the priority, since this can lead to the disappearance/reduction of the inhibitor **(Grade 2C recommendation)**.
- In patients with AHA and clinically significant bleeding, bypassing agents (APCC or rFVIIa) are the first-line treatment **(Grade 1B recommendation)**.
- If the initial bypassing agent is ineffective, the switch to treatment with the alternative agent should be considered at an early stage **(Grade 2C recommendation)**.
- The use of FVIII concentrates and DDAVP should be reserved to patients with measurable FVIII plasma levels and low inhibitor titres. In these cases the initial dose of FVIII must be sufficient to overcome the inhibitor and provide an adequate haemostatic level. It is, therefore, always appropriated to evaluate the adequacy of the treatment by measuring FVIII levels after administration of the initial dose and, subsequently, regular monitoring the FVIII level, at least daily. This is also useful to detect the

development of a possible anamnestic response **(Grade 2C recommendation)**.

- Tranexamic acid could be useful in cases of mucosal bleeding, alone or in addition with other haemostatic agents **(Grade 2B recommendation)**. The only contraindication is the presence of renal tract bleeding **(Grade 1B recommendation)**. Caution, however, should be adopted when tranexamic acid is used in association with activated clotting factor concentrates, in particular with APCC and in elderly patients or in those with comorbidities associated with a high thromboembolic risk **(Grade 2B recommendation)**.
- Plasmapheresis and/or immunoadsorption in combination with the use of high dose of FVIII concentrates can, exceptionally, be considered in patients with AHA who require rapid control of bleeding and do not respond adequately to either type of bypassing agent or who require urgent surgery or invasive procedures **(Grade 2B recommendation)**.
- DDAVP or FVIII/VWF concentrates are indicated for the treatment of minor bleeding in patients with AVWS. The levels of FVIII and VWF should be monitored after the treatment because the half-lives of these clotting factors can be very short in patients with acquired inhibitor antibodies **(Grade 2C recommendation)**.
- Fibrinogen concentrates are the first-line treatment in patients with acquired anti-FI inhibitor. Patients should be monitored frequently during this treatment in order to determine the levels of fibrinogen achieved **(Grade 2C recommendation)**.
- The suggested first-line treatment in patients with acquired anti-FII and anti-FX inhibitor is APCC that can overwhelm the inhibitor and provide sufficient haemostatic activity. Alternatively PCC may be considered **(Grade 2C recommendation)**.
- Fresh-frozen plasma and transfusion of platelet concentrates are the main therapeutic option in patients with acquired anti-FV inhibitor. A possible alternative is APCC, especially in patients at high risk of cardiovascular complications, given the additional risk deriving from the circulatory overload if large volumes of plasma need to be infused **(Grade 2C recommendation)**.
- APCC is the suggested treatment of first-choice in patients with high-titre, acquired anti-FVII inhibitor. If this is ineffective, especially in patients with low inhibitor titres, the use of rFVIIa or plasma-derived FVII concentrate may be considered. The initial dose should be sufficient to overcome the inhibitor and provide an adequate haemostatic level. Subsequently, the level of FVII must be monitored closely in order to adjust the dose and frequency of administration until effective haemostasis is achieved and maintained **(Grade 2C recommendation)**.
- In patients with acquired anti-FIX or anti-FXI inhibitor, the suggested treatment of choice is the use of bypassing agents (APCC or rFVIIa) **(Grade 2C recommendation)**. Other options include FIX and FXI concentrates, respectively, particularly in patients with low-titre inhibitors, or in case of inadequate response to bypassing agents; the level of factor reached must be monitored carefully in order to adjust the dose and frequency of administration so that the neutralising effect of the inhibitor can be overcome and effective haemostasis can be provided and maintained **(Grade 2C recommendation)**.
- Large doses of plasma-derived or recombinant FXIII concentrates are the only therapeutic option in patients with acquired anti-FXIII inhibitor. Carefully monitoring of the plasma levels achieved is indicated in order to adjust the doses and assure effective haemostasis **(Grade 2C recommendation)**.
- In patients with acquired inhibitor against any other clotting factor who do not respond to the previously indicated first-line treatment, an early switch to the second-line treatment is always indicated **(Grade 2C recommendation)**.
- Plasmapheresis and/or immunoadsorption can be considered in patients with acquired inhibitor against any clotting factor (in the same way as indicated for patients with AHA) who have severe bleeding and fail to respond to first-line treatment or require urgent surgery or invasive procedures **(Grade 2C recommendation)**.
- The use of fresh-frozen plasma is contraindicated, whatever the specificity of the inhibitor, except in patients with acquired anti-FV inhibitor, because the large volumes needed to overwhelm the neutralising effect of the inhibitor would create a high risk of circulatory overload **(Grade 2C recommendation)**.

The evidence reported in the literature enables the following recommendations concerning immunosuppressive treatment to eradicate inhibitors in patients with AHA:

- Immunosuppressive treatment must be started as soon as possible, ideally immediately after the diagnosis has been made **(Grade 1B recommendation)**.
- The first-line treatment is oral prednisone at a daily dose of 1-2 mg/kg either alone or in combination with oral cyclophosphamide at a daily dose of 1-2 mg/kg **(Grade 1B recommendation)**.
- The use of cyclophosphamide and other alkylating agents should be avoided in fertile women **(Grade 2C recommendation)**.

- Rituximab (375 mg/m$^2$ once a week for four doses overall) may be indicated as first-line therapy in patients with contraindications to the use of standard immunosuppressive drugs **(Grade 2B recommendation)**.
- Rituximab, alone or in combination with immunosuppressive drugs, is the main component of second-line treatment in the case of lack of response to first-line treatment within 8-12 weeks **(Grade 2B recommendation)**.
- The combination of several immunosuppressive drugs (including cyclosporine) and immune tolerance induction are alternative options in the case of failure to first-line immunosuppressive therapy **(Grade 2C recommendation)**.
- The use of HDIg is not indicated as a treatment for inhibitor eradication **(Grade 1B recommendation)**.
- A persistent undetectable inhibitor (<0.6 UB/mL) with normal plasma levels of FVIII (>70%) is the criterion for the definition of complete response to eradication therapy **(Grade 2B recommendation)**.
- Patients with risk factors for thromboembolism should receive mechanical and/or pharmacological thromboprophlyaxis, particularly in case of excessively high levels of FVIII during/at the end of eradication therapy **(Grade 2C recommendation)**.

For patients with acquired inhibitors against other clotting factors, and for patients with AVWS, the following recommendations concerning immunosuppressive treatment can be made:

- The use of immunosuppressive therapy is not always indicated, since many inhibitors are transient and do not cause significant bleeding. In selected cases a "wait and watch" approach may be indicated, taking into account that the resolution of a concomitant neoplastic or autoimmune disease can give rise to spontaneous remission of the inhibitor **(Grade 2C recommendation)**. In particular, treatment of the underlying disease must be considered the first approach in patients with AVWS and antibody inhibitors **(Grade 1C recommendation)**.
- If bleeding symptoms prompt starting immunosuppressive therapy, the same criteria defined for patients with AHA can be applied **(Grade 2C recommendation)**.
- If immunosuppressive therapy is not indicated or the antibody persists, especially if belonging to IgG class, treatment with HDIg should be considered. The response to treatment should, however, be monitored and, if positive, its duration assessed by a test infusion of HDIg (1 g/kg/die for 2 days or 400 mg/kg/die for 5 days), measuring FVIII and VWF levels at least 1, 7 and 15 days after the end of the first cycle of treatment. Furthermore, the treatment should be prolonged, usually being administered at intervals of about 21 days (even only 1 g/kg/die), especially in patients in whom the risk of bleeding remains high (e.g., in patients with gastrointestinal bleeding due to angiodysplasia) **(Grade 2C recommendation)**.

The following general recommendations apply to all patients with AHA and patients with acquired inhibitors against any other clotting factor:

- Patients with acquired inhibitors against any clotting factor must be managed by specialised Centres for the care of haemophilia and other bleeding disorders, with expertise in the treatment and laboratory monitoring of patients with inhibitors **(Grade 1B recommendation)**.
- Invasive procedures must be avoided in patients with a suspected acquired inhibitor until the diagnosis has been clarified **(Grade 1B recommendation)**.
- Once remission of the inhibitor has been achieved, patients must continue to be monitored for at least 12 months, because there is a significant risk of recurrence **(Grade 1C recommendation)**.
- In patients with a previous acquired inhibitor, the factor affected by the inhibitor should always be assayed before surgery or any invasive procedure **(Grade 2C recommendation)**.

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**Correspondence:** Antonio Coppola
Regional Reference Centre for Coagulation Disorders
Federico II University Hospital
Via S. Pansini 5
80131 Napoli, Italy
e-mail: antocopp@unina.it

## Appendix

**List of co-Authors (in alphabetical order)**

- Rosanna Abbate (Firenze)
- Anna Brigida Aru (Cagliari)
- Chiara Biasoli (Cesena)
- Alessandra Borchiellini (Torino)
- Teresa Maria Caimi (Milano)
- Isabella Cantori (Macerata)
- Anna Maria Cerbone (Napoli)
- Simone Cesaro (Verona)
- Carlo Ciabatta (Latina)
- Antonella Coluccia (Scorrano)
- Laura Contino (Alessandria)
- Dorina Cultrera (Catania)
- Raimondo De Cristofaro (Roma)
- Grazia Delios (Ivrea)
- Matteo Nicola Dario Di Minno (Napoli)
- Alfredo Dragani (Pescara)
- Cosimo Pietro Ettorre (Bari)
- Giulio Feola (Vallo della Lucania)
- Gabriella Gamba (Pavia)
- Giorgio Gandini (Verona)
- Anna Chiara Giuffrida (Verona)
- Gaetano Giuffrida (Catania)
- Paolo Gresele (Perugia)
- Hamisa Jane Hassan (Roma, ISS)
- Giuseppe Lassandro (Bari)
- Caterina Latella (Reggio Calabria)
- Silvia Linari (Firenze)
- Matteo Luciani (Roma)
- Maria Elisa Mancuso (Milano)
- Emanuela Marchesini (Perugia)
- Renato Marino (Bari)
- Davide Matino (Perugia)
- Maria Gabriella Mazzucconi (Roma)
- Maria Messina (Torino)
- Marta Milan (Padova)
- Angelo Claudio Molinari (Genova)
- Mariasanta Napolitano (Palermo)
- Lucia Dora Notarangelo (Brescia)
- Flora Peyvandi (Milano)
- Berardino Pollio (Torino)
- Paolo Radossi (Castelfranco Veneto)
- Federica Riccardi (Parma)
- Silvia Riva (Milano)
- Gianna Franca Rivolta (Parma)
- Gina Rossetti (Trento)
- Rita Carlotta Santoro (Catanzaro)
- Maria Luisa Serino (Ferrara)
- Mario Schiavoni (Scorrano)
- Michele Schiavulli (Napoli)
- Piercarla Schinco (Torino)
- Luigi Piero Solimeno (Milano)
- Gianluca Sottilotta (Reggio Calabria)
- Roberto Targhetta (Cagliari)
- Annarita Tagliaferri (Parma)
- Sophie Testa (Cremona)
- Angela Todisco (Monopoli)
- Marina Turello (Udine)
- Lelia Valdrè (Bologna)