Title	Biology of haemostasis disorders
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Aim

Haemostasis comprises all the mechanisms which prevent spontaneous bleeding and stop haemorrhages by repairing the breach in the vascular system.

The National Salaried Workers' Health Insurance Fund (CNAMTS) asked HAS to assess the value of the different laboratory tests for haemostasis abnormalities with a view to updating the section in the Nomenclature of Procedures in Laboratory Medicine (NABM) containing the procedures in laboratory medicine for measuring abnormalities of haemostasis (subsection 5-02). Those tests are: the bleeding time test (Duke's test, Ivy's incision test or Ivy's 3-point test), the thrombin time and correction of the thrombin time, the photometric platelet aggregation test, the test for antibodies to platelet factor 4 where heparin-induced thrombocytopenia type II is suspected, a test for and titration of antihaemophilic factor inhibitors, a test for and identification of the lupus anticoagulant, a test for the G1691A mutation in the factor V (Leiden factor) gene and the G20210A mutation in the factor II gene when testing for risk factors for venous thromboembolism.

Conclusions and results

<u>For the bleeding time (BT)</u>

The bleeding time (BT), an NABM procedure, is a global test to investigate primary haemostasis in vivo which consists in measuring the time needed for bleeding to stop after a superficial incision is made in the patient's skin. Historically, the BT was used to assess haemostasis preoperatively and to investigate a haemorrhagic syndrome.

After a critical analysis of the literature (14 documents consisting of 10 guidelines, one study on the diagnostic value of the test, one position paper and two systematic reviews) and after taking account

of the comments of the three reviewers, it turns out that the BT is not recommended either for the preoperative assessment of the risk of haemorrhage or for the diagnosis of von Willebrand's disease, and consequently has no place in the diagnostic strategy. Moreover, there are alternatives which are already NABM procedures. In conclusion, BT is a procedure which should no longer be used in current practice (deletion proposed).

<u>For the thrombin time (TT) and the corrected thrombin time</u>

The thrombin time (TT), an NABM procedure, is a global test for the investigation of fibrin formation. The TT is the coagulation time at 37°C of citrated platelet-poor plasma (PPP) in the presence of thrombin. Clot formation is detected by chronometric, electromechanical or optical methods. A test of correction by adding control plasma can be carried out: the absence of any correction in TT indicates that unfractionated heparin is present in the sample. Historically, TT was used to make an assessment of preoperative haemostasis, investigate a haemorrhagic syndrome or to detect the presence of unfractionated heparin in the sample.

After a critical analysis of the literature (nine guidelines on diseases associated with the prescribing circumstances but no document recommending TT) and after taking account of the comments of the three reviewers, it emerges that the TT is not advisable either for the preoperative assessment of the risk of haemorrhage or for the investigation of a haemorrhagic syndrome. Moreover, there is an alternative which is already an NABM procedure (determination of fibrinogen). In conclusion, TT is a procedure which should no longer be used in current practice (deletion proposed).

For the photometric platelet aggregation test (PAT):
The PAT, which is not an NABM procedure, is regarded as the reference test for the assessment of platelet function, for which it continues to be the most used test. This technique measures aggregation in platelet-rich plasma (PRP), obtained after low-speed centrifugation of the patient's blood sample, collected in sodium citrate. Aggregation is measured by a photometric technique after the addition of various inducers, also called aggregating agents or agonists.

After a critical analysis of the literature (15 documents comprising 10 guidelines, one study on the diagnostic value of the test, one HAS technological assessment report from 2005 and three position papers), and after taking account of the comments of the three reviewers, it can be concluded that the PAT should be used for the diagnosis of abnormalities of platelet function and for the laboratory diagnosis of HIT in combination with an immunological test (inclusion of the PAT proposed). Testing for resistance to antiplatelet drugs is not currently recommended for routine individual use by the literature identified. Since the preanalytical and analytical requirements are fairly demanding, this test needs to be carried out at experienced centres that are sufficiently busy to maintain expertise in using the technique and interpreting the results.

For testing for antibodies to platelet factor 4 (PF4)
This test by an immunological method (ELISA in particular), which is not an NABM procedure, is, along with the functional tests (including the PAT already mentioned), one of the two types of tests that can be used to detect heparin-induced thrombocytopenia. HIT is a serious complication of the parenteral anticoagulant treatments that are generally used for the prevention and treatment of venous thromboembolism (VTE). HIT is responsible for arterial or venous thromboembolic accidents that can be life-threatening or jeopardize patients' functional prognosis.

After a critical analysis of the literature (five documents comprising four guidelines, and one HAS technological assessment report from 2005), and taking account of the comments of the three reviewers, it can be concluded that testing for anti-PF4 antibodies is recommended if there is any suspicion of HIT which is a serious complication of parenteral anticoagulant treatments (inclusion proposed). According to the French guidelines analysed, it is essential to combine a functional test (for example the PAT) with an immunological test (ELISA). This allows HIT to be diagnosed on the basis of a body of arguments and treatment to be adjusted in the short and long term (change of treatment, secondary prevention).

For testing for and titration of inhibitors of antihaemophilic factors (AHF)
Congenital haemophilia is a haemorrhagic disorder linked to a deficiency in antihaemophilic factor (AHF): factor VIII (haemophilia A) or factor IX (haemophilia B). The commonest and most dreaded complication is the appearance of antibodies which inhibit the procoagulant activity of AHF, mainly as a result of severe haemophilia A.

After a critical analysis of the literature (seven guidelines including five detailing research into AHF inhibitors and two on the management of haemophiliac patients with inhibitors) and taking account of the comments of the three reviewers, it can be concluded that testing for and titration of AHF inhibitors by the Bethesda-Nijmegen method is recommended in the diagnosis, management and follow-up of the treatment of haemophiliac patients (inclusion proposed).

For the detection of the lupus anticoagulant
Venous thromboembolism (VTE), the two main forms of which are deep-vein thrombosis (DVT) and pulmonary embolism (PE), is a complex disorder resulting from the interaction of numerous genetic and environmental risk factors which constitute an individual's predisposition to thrombotic events. Numerous studies have shown a link between VTE and certain biological risk factors (BRFs), including antiphospholipid syndrome (APLS).

After a critical analysis of the literature (six documents including four guidelines, one HAS technological assessment report from 2006 and a position paper) and taking account of the comments of the three reviewers, it can be concluded that detection of the lupus anticoagulant is necessary as part of the diagnosis of APLS and can be used to adjust patients' treatment (inclusion proposed). The lupus anticoagulant can be detected in three stages: screening (dilute Russell's viper venom time and activated partial thromboplastin time using silica), demonstration of an inhibitory effect (mixing stage), and confirmation (neutralisation stage). Detection of

the lupus anticoagulant must be supplemented by a test for anti-cardiolipin and anti-β2GP 1 antibodies.

For testing for the G1691A mutation in the factor V gene (Leiden FV) and the G20210A mutation in the factor II gene (prothrombin)

These two mutations are genetic variants, each due to a point mutation of the corresponding genes; they have been described as risk factors for VTE. These two mutations are identified by the methods used to test for point mutations (the reference technique for which is still bidirectional sequencing); they can be used to identify mutations and determine their type (homozygous or heterozygous).

After a critical analysis of the literature (eight documents including five guidelines, two technological assessment reports including ones published by HAS in 2006 and one experts report on genetic tests in general) and taking account of the comments of the three reviewers, it turns out that these two mutations are among the biological risk factors for VTE, even though their predictive value is poorly defined. It also emerges that testing for them is suggested in specific clinical situations where there is a risk of the recurrence or onset of VTE:

- occurrence of unprovoked VTE before age 50/60 years or of provoked or unprovoked VTE in pregnant women;
- recurrence of proximal DVT and/or PE, or of unprovoked distal DVT the first episode of which occurred before age 50/60 years;
- in pregnant women with a family history of VTE.
- after a case-by-case discussion:
- in pregnant women with a family history of hereditary thrombophilia.
- in pregnant women with a history of multiple spontaneous abortions or unexplained intrauterine fetal death, preeclampsia, HELLP syndrome, premature detachment of the placenta or fetal growth retardation;
- in cases where there is a family history of VTE in a first-degree relative with homozygosity or double heterozygosity of mutations of FV and FII, in women of child-bearing age before the prescription of oestrogen-progestogen contraception.

In these situations, it can be concluded that identification of the G1691A mutation in the gene for factor V and of the G20210A mutation in the gene for factor II should be suggested when checking for risk factors for VTE (inclusion proposed). It should be noted that the analysis of a person's genetic characteristics for medical purposes, the conditions for prescribing these procedures, carrying them out in practice, the conditions for communication of the result and the conditions for storage of the documents must be regulated and must comply with the legislation in force (PHC Articles L. 1131-1 ff and R. 1131-1 ff). These examinations can be performed only by laboratories authorised to carry out genetic tests.

Recommendations

In conclusion, the deletion of four tests is proposed (Duke's test and Ivy's test for the bleeding time and the thrombin time and correction of the thrombin time), and six tests are proposed for inclusion in the Nomenclature of Procedures in Laboratory Medicine (NABM): photometric platelet aggregation test, test for antibodies to platelet factor 4, test for and titration of antihaemophilic factor inhibitor, test for and identification of the lupus anticoagulant, test for the G1691A mutation in the gene for factor V and test for the G20210A mutation in the gene for factor II.

Methods

This assessment is based on a critical analysis of the literature made by the Haute Autorité de Santé, and reviewed by experts in haemostasis. It takes into account the arguments of the group of experts assembled by CNAMTS on which CNAMTS based its request. The search covered the subjects and types of studies defined in the CNAMTS request and was limited to publications in English and French. For guidelines and reviews about the bleeding time, the search was started with no time limit and continued until May 2011. For guidelines about the thrombin time, the search was started with no time limit. For reviews about the thrombin time, the search was started in January 2000. For this procedure, an additional search was made, without the keywords thrombin time, for the diseases for which the thrombin time could be used, and for the determination of fibrinogen. For the other techniques, the search started in January 2000. For all these techniques, the search continued until May 2011. The following sources were interrogated: the Medline database, the Pascal database and the Public Health Database and the Cochrane Library; websites publishing guidelines, technological or economic assessment reports; and the websites of the learned societies relevant to the field studied. A

total of 1210 references were identified, 499 were analysed, and 49 were selected. Three experts reviewed and commented on the report without giving a verdict on HAS's final conclusions. Their comments were collected in writing, then discussed. Once validated, the comments were included either directly in the body of the text or in a dedicated section.

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