HEALTH TECHNOLOGY ASSESSMENT OF GENETIC TESTING FOR SUSCEPTIBILITY TO VENOUS THROMBOEMBOLISM IN ITALY

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CHAPTER 1: INTRODUCTION

1.1 VENOUS THROMBOEMBOLISM (VTE): THE CLINICAL CONTEXT

Venous thromboembolism (VTE) is a condition in which a thrombus (a solid mass of blood constituents) forms in a vein. VTE represents an extremely common medical problem manifested as either deep venous thrombosis (DVT) or pulmonary embolism (PE) affecting apparently healthy as well as hospitalized patients. Often PE is the physiopathological consequence of the DVT of low extremities vessels, in particular of the calves (1).

The pathogenesis of VTE was described over 150 years ago by Rudolph Virchow and summarized in the well-known triad describing the three necessary components for thrombosis: a) blood stasis, b) hypercoagulability, and c) changes in the vessel wall (2).

Thrombophilia is a defect in blood coagulation that leads to a predisposition towards thrombosis. It can be heritable (genetic) or acquired (or mixed). Heritable (genetic) thrombophilia is caused most commonly by mutations in the genes for coagulation factors II and V. Factor V Leiden (FVL) is the most common known inherited risk factor for thrombosis, resulting from the G>A substitution at position 1691 of the gene encoding coagulation Factor V (R506Q). FVL causes the Factor V being inactivated more slowly and generating more thrombin. Prothrombin 20210G>A (PT20210A), the second most common known inherited risk factor for thrombosis, produces an amino acid substitution which results in higher circulating prothrombin levels. Therefore, both FVL and PT20210A enhance the potential for clot formation.

Acquired thrombophilia refers to conditions in which individuals without genetic defects in coagulation factors are at increased risk of thrombosis, for example those with lupus anticoagulant or anticardiolipin antibodies. Examples of mixed type thrombophilias are elevation of factor VIII or homocysteine levels. As homocysteine level in plasma is in part under control of the methylenetetrahydrofolate reductase, coded by the MTHFR gene, its functional variant C677T has been considered a relevant risk factor for VTE and included in a panel of genetic tests for inherited thrombophilia comprising also FVL and PT20210A (3).

Many intrinsic factors, disease-related risk factors and physiological or iatrogenic factors (e.g., pregnancy, oral contraceptives and hormone replacement therapy) can increase the propensity to VTE. Among intrinsic risk factors age, obesity, genetic factors and a previous history of VTE are the main predictors of VTE. Advanced age is associated with an increased risk of VTE with a reported cumulative probability of experiencing a first case of VTE at age 80 years 20-fold higher than at age 50 years (4). Obesity, in particular abdominal obesity, has been found as an independent factor associated with VTE that increases two times the risk in subjects with a body mass index (BMI) greater than 30 kg/m² (5).

A previous event of venous thromboembolism is considered the most important factor associated with VTE, increasing the risk of recurrent events of about 15 times (6). Surgical procedures (in particular orthopedic surgery, surgery for cancer and neurosurgery) are the most relevant disease-related risk factors for VTE. It has been found that, under surgical interventions, incidence of thromboembolic events can vary from 15% to 60%, with the higher frequency detected among patients undergoing hip or knee arthroplasty and hip fracture surgery (7).

The clinical relevance of VTE is highlighted by the significant rates of recurrence and mortality that, however, are very likely to be underestimated since relevant epidemiological data for the frequency of VTE derive mainly from large community-based studies that reflect symptomatic rather than asymptomatic disease.

1. 2. GENETIC TESTING: METHODOLOGY OF EVALUATION

After the complete and comprehensive sequencing of the human genome, the development and utilization of genome-based tests are rapidly expanding. Currently, genetic tests for more than 1 800 diseases are available and this growing number of tests may be used in order to
HTA of Genetic Testing for Susceptibility to VTE

I. Introduction

The current HTA project was conducted in the context of a national multicenter project titled “Health Technology Assessment for genetic screening: genetic testing for susceptibility to venous thromboembolism as a case study”. This was funded from the Ministry of University within the context of the National Projects of Interests (PRIN) in 2007. Members of the working group belong to the Italian Network of Public Health Genomics (Network Italiano di Genomica in Sanità Pubblica, GENISAP, http://istituti.unicatt.it/igiene_1830.html), funded in 2006 at the Institute of Hygiene in the Faculty of Medicine of the Università Cattolica del Sacro Cuore, Rome, Italy (UCSC) in Rome (15). The GENISAP was created as a follow-up of the European Network of Public Health Genomics (http://istituti.unicatt.it/igiene_1830.html) and it is coordinated by Walter Ricciardi (Director of the Institute of Hygiene-UCSC) and Stefania Boccia. The aim of GENISAP is to integrate genomics into public health policy and practice in Italy in a responsible and affective manner.

II. The Purpose of the Work

The purpose of our work is to carry out an HTA project on genetic testing for susceptibility to VTE in Italy. In Italy the screening for inherited thrombophilia accounts for 28% of all genetic tests performed in adults, with a growing trend in recent years (13, 14).

III. Methods

This HTA report aims to assess the current scenario of genetic testing provided for the susceptibility to VTE in Italy. The project is based on the ACCE (Analytic validity, Clinical validity, Clinical utility, Ethical, legal and social aspects) model, which was developed by the National Office of Public Health Genomics, CDC in 2004 (16). By using a comprehensive approach, the report considered all the possible implications related to the utilization of genetic testing for the susceptibility to VTE. As such, the report can be used as a tool for providing an up-to-date evidence on the topic, to inform policy-makers, citizens, physicians and all the stakeholders, in order to adequately support the decision making process.

IV. References

(2) Virchow R. Gesammelte Abhandlungen zur wissenschaftlichen Medizin. Frankfurt, Germany: Medinger Sohn, 1856
(3) Moheimani F, Jackson DE. Venous thromboembolism: classification, risk factors, diagnosis, and management. ISRN Hematol. 2011; Epub 2011 Oct 17
Conference on antithrombotic and thrombolytic therapy. Chest 2004; 126 Suppl 3: S388-S408


2.1 Epidemiology of venous thromboembolism and inherited thrombophilia in the Western countries and in Italy

Valerio De Stefano, Tommaso Za, Angela Ciminello, Silvia Betti, Elena Rossi

2.1.1 INCIDENCE OF VENOUS THROMBOEMBOLISM IN THE UNITED STATES

Epidemiological data concerning the incidence of venous thromboembolism (VTE) have been obtained from community studies and hospital discharges; the former approach is likely to include also those patients managed on an outpatient basis, reducing underestimation. Most of the data on the incidence of VTE is provided by studies which were performed either in United States (1-5) or in Northern Europe (6-11).

The seminal population-based Tecumseh Community Health Study was conducted in a Michigan community from 1959 to 1969, and estimated an overall incidence per 1 000 persons-years of 0.22 for pulmonary embolism (PE), 1.17 for deep venous thrombosis (DVT), and 0.47 for superficial venous thrombosis (SVT). The first events quoted for an incidence per 1 000 persons-years of 0.15 for PE, 0.87 for DVT, and 0.28 for SVT (1). VTE was predominant in older age: in the Tecumseh Study the incidence of first DVT per 1 000 persons-years was 0.44 for males and 1.48 for females under 46 years of age, and 2.19 for males and 2.69 for females older than 46 years of age (1).

Such data were basically confirmed in subsequent studies. In Minnesota, the medical records of all residents of Olmsted County who were diagnosed with VTE between 1966 and 1990 were analyzed: the average incidence of VTE per 1 000 persons-years was 1.17; the incidence of DVT alone was 0.48 and the incidence of PE (with or without associated DVT) was 0.69. Incidence rates increased markedly with age for both males and females, and for both DVT and PE, starting from 0.003 (females)–0.01 (males) per 1 000 persons-years for residents under 15 years of age and reaching an overall incidence of VTE of 9.65 (females)–9.19 (males) for those residents older than 85 years (2). In this setting a nested case-control study demonstrated that independent risk factors for VTE included surgery (odds ratio, OR, 21.7), trauma (OR 12.7), hospital or nursing home confinement (OR 8.0), malignant neoplasms with (OR 6.5) or without (OR 4.1) chemotherapy, central venous catheter or pacemaker (OR 5.6), SVT (OR 4.3), and neurological disease with extremity paresis (OR 3.0) (3). Fifty-nine percent of the cases of VTE in the community could be attributed to institutionalization (current or recent hospitalization or nursing home residence). Of note, hospitalization for medical illness and hospitalization for surgery account for almost equal proportions of VTE (22% and 24%, respectively) (4).

The Longitudinal Investigation of Thrombosis Etiology (LITE) combined population-based cohorts from two studies: the Cardiovascular Health Study (CHS) and Atherosclerosis Risk in Communities (ARIC) study. Participants from six communities were followed: Forsyth County, North Carolina; Washington County, Maryland; suburban Minneapolis, Minnesota; Jackson, Mississippi; Sacramento County, California; and Pittsburgh, Pennsylvania. Between 1987 and 1989, ARIC enrolled 15 792 subjects aged 45 to 64 years (4 266 African Americans). In 1989–1990 and 1992–1993, CHS enrolled 5 888 subjects older than 65 years (924 African Americans). Thrombosis events were identified through December 31, 1996, in ARIC, and through June 30, 1997, in CHS. The incidence of first VTE per 1 000 persons-years was 1.61, 1.17 for DVT alone and 0.45 for PE (with or without associated DVT). The incidence of first VTE increased with age, reaching above 75 years of age 5.5 in men and 2.7 in women. Half cases of VTE (52%) were secondary and were associated with one or more underlying conditions (in the
majority of cases cancer, hospitalization, surgery, major trauma) (5).

In conclusion, the overall incidence of first DVT and/or PE per 1 000 persons-years in the United States was consistently estimated to range between 1.02 (1) to 1.61 (5); the rate of VTE increases with age and in more than half cases is associated with a transient risk factor.

2.1.2 INCIDENCE OF VENOUS THROMBOEMBOLISM IN EUROPE

Some community studies have been performed in Europe. In Sweden the incidence of VTE has been estimated in Malmö (6, 7) and in Göteborg (8). In the first study carried out in the city of Malmö in 1987, the incidence of DVT was estimated 1.58 per 1 000 persons-years, rising to 4.7 (males)-4.2 (females) in the individuals older than 60 years of age (6). In a more recent study carried out in Malmö during 1998-2006 the incidence per 1 000 persons-years was 0.51 for DVT and 0.19 for PE. The reason of such a decreasing incidence of VTE could be improved thromboprophylaxis in different risk situations; moreover in this latter study autopsy data were not included, that might have contributed to higher incidences in previous studies (7). In the “Study of Men Born in 1913” conducted in Göteborg, 855 men were followed prospectively from the age of 50 years to the age of 80 years: the incidence of VTE per 1 000 persons-years in this cohort was 3.10 for the first events (1.38 for DVT and 1.72 for PE) and 3.87 for all first and recurrent events (1.82 for DVT and 2.05 for PE), with a cumulative probability for a venous thromboembolic event of 0.5% by the age of 50 years and 10.7% by the age of 80 years (8).

Another two studies from Norway estimated in the communities of Nord-Trøndelag (9) and Tromso (10) incidences of VTE similar to those found in the studies from North America. In the county of Nord-Trøndelag the incidence of first VTE per 1 000 persons-years was estimated 1.43 between 1995 and 2001, namely 0.93 for DVT and 0.50 for PE (9). In the city of Tromso the incidence of first VTE per 1 000 persons-years was estimated 1.40 between 1994 and 2007. Cancer was the most common provoking factor (22% of the patients with VTE); subjects 70 years or older had a 11-fold higher risk of VTE compared to those younger than 50 years of age (10). Finally, in the Copenhagen City Heart Study the incidence of first VTE per 1 000 persons-years was 2.69 between 1976 and 2007; in this study obesity and smoking were found to be important risk factors for VTE (11). In conclusion, in most studies from Scandinavian countries the incidence of first VTE per 1 000 persons-years was about 1.5 (6, 9, 10).

In France the incidence of VTE has been studied during one year (1998-1999) in a defined population living in the Brest District in Western France. This study estimated an overall annual incidence per 1 000 inhabitants of 1.83, namely 1.24 for DVT and 0.60 for PE (with or without DVT); the incidence of first VTE was 1.36 per 1 000 persons-years. Over the age of 75 years, the incidence reached 1 per cent. Overall, this estimate is consistent with those found in communities from North America and Northern Europe (12).

The incidence of VTE in the population of United Kingdom was estimated using the General Practice Research Database (1994-2000). The overall incidence rate of VTE was 0.74 per 1 000 persons-years; 29% of cases presented with one of the following risk factors: surgery, fracture in the month preceding diagnosis, cancer. Hospitalization in the previous year was present in 46% of DVT cases and 56% of PE cases, producing a 6.6-fold increased risk in respect to the control population. (13).

A study group proposed an epidemiological model to estimate the number of incident and recurrent VTE events within six countries of the European Union (France, Germany, Italy, Spain, Sweden, and the United Kingdom). According to this model, the incidence per 1 000 persons-years was estimated across the six European countries aforementioned to be 1.48 for DVT and 0.95 for PE (14).

The available data concerning the annual incidence of first VTE in North America and in Europe are summarized in Figure 2.1; studies reporting the overall incidence of VTE without specifying the incidence of first events are not included in the Figure.

2.1.3 PREVALENCE OF VENOUS THROMBOEMBOLISM IN ITALY

Reliable data on the local incidence of VTE in Italy are lacking, because no community-based study focused on this aim has been performed.

A large cross-sectional investigation has been performed in 1993 to 1997 in the town of Vicenza,
The Vicenza Thrombophilia and Atherosclerosis (VITA) Project was aimed to determine the prevalence of first non-fatal VTE and to identify the clinical determinants of VTE in the active population. The VITA Project collected clinical data and a blood sample from 15,055 Caucasian individuals aged 18-65 living in Vicenza at January 1, 1993, and randomly selected from the census list. Subjects with severe physical or mental disease or with a history of active cancer in the last year were excluded. One hundred and sixteen subjects with at least one episode of non-fatal VTE were identified. The prevalence per 1,000 inhabitants resulted 7.69, namely 6.11 for DVT of lower limbs, 0.19 for DVT of upper limbs, and 1.39 for PE. In the Göteborg cross-sectional investigation of “The Study of Men Born in 1913”, the prevalence of VTE was from 5 per 1,000 at the age of 50 years to 20 per 1,000 at age 67 years. However, the data are not comparable, given the exclusion of females from the Göteborg study, and the exclusion of fatal events from the Vicenza study. A nested case-control study was carried out selecting from the database for each case 10 control patients without VTE. Cancer (OR 2.2), neurological diseases (OR 2.20), and previous hospitalization (OR 2.58) resulted the more important risk factors by multivariate analysis. Comparison of such results with other reports is difficult, because the selected population comprises patients with exclusively medical risk factors.

2.1.4 PREVALENCE OF INHERITED THROMBOPHILIA IN THE GENERAL POPULATION

Inherited deficiency of antithrombin (AT), protein C (PC) and its co-factor, protein S (PS), were the first identified causes of thrombophilia. More recently, two common gene polymorphisms were recognized as additional causes of hypercoagulability: factor V G1691A (factor V Leiden), resistant to the anticoagulant action of activated protein C, and prothrombin G20210A, associated with increased levels of circulating prothrombin. Mild hyperhomocysteinemia is also an established risk factor for thrombophilia.
are detectable in less than 1% of the general population and in less than 10% of unselected patients with VTE (20). Factor V Leiden is present almost exclusively among Caucasians, with a prevalence of 5% in the general population with European ancestry and 18% among patients with VTE (21). In some European areas (Sweden, Alsace, Cyprus) the prevalence of factor V Leiden in the general population has been reported to be 10 to 15% (21). Finally, the prothrombin 20210A allele is present in 2% of healthy individuals and in 7% of patients with VTE (22). Acquired factors (low intake of pyridoxine, cobalamin, folate) can produce mild hyperhcysteinemia interacting with gene factors, such as the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene. Homozygous carriers can develop hyperhomocysteinemia especially in the presence of low folate levels. Among Caucasians the prevalence of the TT genotype is 13.7%, quite similar to that found among patients with VTE, suggesting that search for this genotype is not useful per se (23). Such figures have been recently confirmed in a large case-control Dutch study (Multiple Environmental and Genetic Assessment, MEGA, of risk factors for VTE study), recruiting 4,375 patients and 4,856 control subjects. Among those latter, the frequency of factor V Leiden, prothrombin G20210A, heterozygous MTHFR C677T, and homozygous MTHFR C677T was 5%, 2%, 43%, and 11%, respectively. In the patients the distribution of factor V Leiden, prothrombin G20210A, heterozygous MTHFR C677T, and homozygous MTHFR C677T was 16%, 5%, 43%, and 10%. Accordingly, factor V Leiden and prothrombin G20210A were confirmed to be risk factors for VTE, whereas the carriership of the MTHFR C677T polymorphism had null effect on the risk for VTE (24).

The prevalence of inherited thrombophilia in a large Italian population was investigated in the frame of the VITA study. The prevalence of factor V Leiden was found 2.4% for heterozygotes and 0.1% for homozygotes (15,16). This fits with other Italian studies of smaller size which reported a prevalence of factor V Leiden in the general population between 2.6% and 3.0% (25-27). In the VITA study heterozygosity for prothrombin G20210A was found in 4.3% of the cases with VTE and 3.4% of the population without VTE; no homozygous carrier was found. In contrast with most studies, the presence of the G20210A allele had only a marginal effect on the risk for VTE (17). Finally, the prevalence of homozygotes for the MTHFR C677T polymorphism was 12.3% among the patients with VTE and 13.8% among the controls (15).

Notably, in a small-size study from Reggio Calabria, in the Southern Italy, the prevalence of factor V Leiden and prothrombin G20210A among blood donors was found 9.5% and 5.7%, respectively, higher than that usually observed in studies from centers located in Northern and Middle Italy. The authors speculated that this could be consequent to the ancient Phoenician and Greek colonization (28). However, another study from Chioggia, in the Northern Italy, not far from Venice, reported a high prevalence of 11.4% heterozygotes and 0.4% homozygotes for factor V Leiden among 471 healthy subjects (29). The possible existence of clusters of genes associated with thrombophilia in some Italian areas calls for novel population-based studies aimed to address the prevalence of inherited thrombophilia in the general population, the incidence of VTE, and the fraction of events attributable to inherited thrombophilia in Italy.
2.2 Provision of genetic testing for inherited thrombophilia in Italy

Elisa Giorgio, Vera Uliana, The Project Unit Investigators, Emilio Di Maria

2.2.1 INTRODUCTION

Families with a marked predisposition to venous thrombotic events (VTE) were described since the first part of the 20th century (30). It had been clearly recognised that acquired risk factors, such as surgery and pregnancy, contribute to the occurrence of thrombosis, but also that genetic factors segregating in families determine an increased tendency to undergo a thrombotic event. Patients with genetic thrombophilia exhibit an increased predisposition to recurrent VTE and the thrombotic event usually occurs early in life (before 45 years of age). In up to one third of cases, a family history of thrombosis can be identified. However, the proper interpretation of familial thrombosis was hampered, until recently, by the limited knowledge about the effects of genome variability and its interaction with environmental factors.

The evaluation of heritability of thrombosis liability by a variance component method estimated that more than 60% of the variation is attributable to genetic factors (31). Several quantitative traits exhibited significant genetic correlations with thrombosis, including levels of coagulation factors (factors VII, VIII, IX, XI, XII, and von Willebrand), tissue plasminogen activator, homocysteine, and the activated protein C ratio (31), thus implying that genetic factors that influence quantitative variation in these physiological correlates also influence the risk of thrombosis.

After a diagnosis of VTE, screening for thrombophilia is often prescribed, particularly after an early-onset event, thrombosis at an unusual body site, recurrent thrombosis, or thrombosis during pregnancy. Laboratory evaluation commonly includes testing for activated Protein C resistance, antithrombin, Protein C and Protein S deficiencies, lupus anticoagulant, anticardiolipin antibodies, anti-2-glycoprotein-I antibodies, and Factor VIII levels. Search for mutations in the genes for coagulation factors II and V are the most prescribed genetic tests.

Prothrombin (PT) 20210A, factor V Leiden (FVL) G1691A and ethylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms are the most common inherited risk factors for VTE (see Chapter 1).

In light of the wide use of genetic testing for thrombophilia in clinical practice, a substantial body of literature addressed its appropriateness and several recommendations were produced (32-35), also regarding the quality assurance issues in laboratory procedures (36-38). A systematic review and cost-effectiveness analysis was recently accomplished to evaluate whether thrombophilia testing following a VTE is clinically effective and cost-effective. Notably, the study underscored the lack of primary evidence that lead to a large degree of uncertainty in the final estimations (39).

Though MTHFR genetic testing should not be routinely included in thrombophilia screening according to international literature (38), genotyping of the MTHFR C677T polymorphism is still offered by laboratories as an element of the thrombophilia screening panel. Here we focus on genetic testing for FVL, PT20210A and MTHFR C677T. Testing for other inherited and acquired factors will not be discussed in further detail. Thus, we refer to the combination of FVL, PT20210A and MTHFR C677T as “genetic tests” for thrombophilia from hereafter, if not differently stated.

2.2.2 MATERIALS AND METHODS

The list of laboratories offering genetic tests for thrombophilia was extracted from the data set of the survey on genetic tests in Italy (40) (courtesy of Prof. B. Dallapiccola). Sixty-six laboratories offering at least one thrombophilia genetic test (FVL, PT20210A and MTHFR C677T) were found. An exhaustive search for laboratories in Liguria, by mean of Internet resources, phone directories and personal contacts, provided additional 27 laboratories (out of a list of 52 contacted) offering genetic testing for VTE (3 were listed in the nationwide survey mentioned above (40)). For the present report, which does not entail statistical analysis of data, we considered the entire sample.
CHAPTER 2: PROVISION OF GENETIC TESTING

of laboratories as a whole, with no stratification.

The survey instrument (available on request) was a two-page questionnaire designed in Italian, which took approximately 15 minutes to complete. It was introduced by a letter in which we outlined the aim of the study and the research programme.

The questionnaire was an electronic form comprising 17 items, organized in three main sections: i) general features and organisation of the laboratory (e.g. denomination, private or public ownership); ii) offer of biochemical and genetic tests for thrombophilia (number of tests performed, methods, quality control, etc.); iii) pre-test and post-test procedures (indication to test, pre-test counselling, reporting); the last part of the form included the consent to recontact, additional information on the laboratory and an optional comment field. Most items had predetermined, multiple-choices answers; a few questions required a numeric or yes/no answer; one required an open answer. All data investigated were referred to the year 2008. Answers were exported from the electronic form and collected into one data set for descriptive analysis.

The study questionnaire was sent to all selected laboratories via e-mail. The contact persons were the director of the laboratory or the person in charge for thrombophilia screening. A reminder was sent twice (by phone and e-mail) to those who did not respond after one month. Fifty-two questionnaires were collected (response rate: 52/89, 58.4%); 36 were from laboratories which had participated in the nationwide survey (40).

2.2.3 RESULTS

Forty-five laboratories declared to offer thrombophilia genetic tests. The total count of tests reported by laboratories was 20 293 for FVL, 19 854 for PT20210A and 13 347 for MTHFR C677T (corresponding to 27.9%, 37.1% and 25.0%, respectively), resulting in 53 494 tests. The total number of tests rises to 68 805 if other variants are included (namely Factor V Y1702C and H1299R, MTHFR A1298C, analysed by 7, 20 and 33 laboratories, respectively). On average, these counts correspond to 1 323 thrombophilia genetic tests per laboratory per year.

Considering laboratories located in Liguria (8 offering thrombophilia genetic testing), we counted 8 881 thrombophilia genetic tests per year (4 058 FVL, 3 355 PT20210A and 1 468 MTHFR C677T, respectively), corresponding to 1 110 test per laboratory on average and about 5.5 tests per 1 000 inhabitants per year. Table 2.1 reports the number of laboratories offering testing
for thrombophilia surveyed by Italian region and the number of tests performed.

Laboratories performing each assay individually and laboratories testing all variants in the same assay were equally distributed (22 and 22; 1 no answer). The two most used methods resulted real-time PCR (22 laboratories) and reverse hybridisation (19 laboratories); other methods, such as RFLP, primer extension assay, allele-specific PCR, were quoted by 10 respondents (4 answers missing).

Most laboratories reported to employ a dedicated commercial kit (40/52, 76.9%). The kit control samples are used as reference standard by 48% of laboratories; 28.8% (15/52) use their own control samples; 9.6% (5/52) declared to use certified standard material as analytic control. Twenty-eight laboratories (out of 52, 53.8%) reported to regularly participate into external quality assessment schemes (no: 18/52; no answer: 6/52).

Two items explored the offer of genetic counselling: 44.2% declared that pre-test genetic counselling is regularly offered by the staff of the laboratory; after a positive test result, 36.5 of respondents declared to suggest genetic counselling in the conclusions of the laboratory report, whereas 23% suggest testing in relatives; 30.8% declared to avoid suggestions as the prescribing doctor is responsible for that. Most laboratories (75%) deliver the final report to the patients, rather than to the physician who ordered the test (none to the family doctor).

2.2.4 DISCUSSION

In this section we address a few relevant points to provide an outline of the current provision of thrombophilia genetic testing in Italy (more details on procedures and results will be reported elsewhere [manuscript in preparation]). Prior to our survey, it was already known that genetic testing for VTE represents a substantial part of molecular genetic tests offered in Italy. The latest survey on Italian genetic laboratories, which collected data about the activities accomplished during 2007 (see www.sigu.net), showed that the sum of the assays carried out for FVL, PT20210A and MTHFR C677T is no longer considered an appropriate test for inherited thrombophilia (32, 33, 38).

Consistently with the above mentioned study (40), we may infer that genetic testing for VTE as a whole is the most offered genetic test in Italy, despite the fact that it should be considered a predictive test of susceptibility to VTE, and not a diagnostic test. It is worth to recall that genetic thrombophilia, defined as an inherited condition predisposing to the development of pathologic thromboses, is an example of the modern paradigm of complex disorder, i.e. a disease whose aetiology is determined by multiple interacting genetic and environmental factors. In other terms, carriers of genetic variants associated with inherited thrombophilia have an increased risk to develop VTE; the latter is determined, however, by a number of genetic and non-genetic variables. Therefore, in no circumstance the result from genetic testing alone is sufficient to establish neither the diagnosis nor the prognosis after a VTE episode. Despite this knowledge, genetic testing for VTE is frequently ordered. To what extent the inappropriate request by prescribers depends also on an excess of offer by laboratories, is beyond the scope of our discussion.

Though a certain degree of heterogeneity appeared among different laboratories, consistently with the different clinical and geographical context, we observed some peculiar element shared by almost all surveyed centres. According to the study questionnaire, during 2008 the number of tests on Factor V, Prothrombin and MTHFR, respectively, was approximately equal. The section on methods employed demonstrated that most laboratories use commercial kits, commonly based on real-time PCR or reverse hybridisation assays. To our knowledge, these kits commonly analyse the three most investigated variants in one panel, i.e. include FVL, PT20210A and MTHFR C677T in the same assay. This technical approach likely influences choices of laboratories towards offering the three genetic variants as a single panel of genetic investigation, and may explain the excess of MTHFR C677T tests. Whether clinical utility of testing FVL and PT20210A is still questionable, and strongly depends on the clinical context as well as on parameters included in analysis (39, 41), it is quite clear that MTHFR C677T is no longer considered an appropriate test for inherited thrombophilia (32, 33, 38).

Furthermore, we showed that several laboratories suggest cascade testing, that is testing in relatives after a positive test in the index patient. Recent recommendations stated that
there is no evidence in favour of clinical utility of testing FVL or PT20210A in asymptomatic family members of patients with VTE (32). Recently, consensus statements reporting the evidence-based international recommendations were published in Italy (42). We might expect that the effect of information on recommended practices, based on the body of international and Italian literature, will be apparent in the near future.

We are aware that this survey carries an intrinsic inclusion bias, as responders were enrolled from previous surveys endorsed by the Italian Society of Human Genetics (see www.sigu.net and (40)). Furthermore, the sample is rather limited and cannot be considered representative of all public and private Italian laboratories offering genetic testing for VTE. However, at least one regional area, i.e. Liguria, was exhaustively surveyed. By comparing results from Liguria with those from the rest of Italy, we did not observe striking differences in terms of procedures (data not shown). Therefore, we believe that data collected in Liguria may be a reliable example of the current practice in Italy, at least concerning the number of test per year per inhabitant.

A few possible caveats may be suggested based on the survey results. Though addressing analytic validity of genetic testing for VTE is beyond the scope of the present report, we would underscore that almost half of the surveyed laboratories did not mention their participation to external quality assessment schemes. Moreover, a minority of them currently use reference standard material as analytic control.

The study questionnaire suggested heterogeneous attitudes towards genetic counselling, which is systematically offered to a minority of patients. As genetic counselling is crucial for a complete evaluation of the actual genetic testing procedure, its offer was explored by mean of a dedicated survey on patients who underwent genetic testing for VTE, and will be specifically addressed elsewhere [manuscript in preparation].

The current use of thrombophilia genetic testing as outlined above raises a relevant issue of appropriateness, which in turn relies on accurate assessment of clinical validity and clinical utility. The latter should be evaluated in the clinical context, taking into account the specific clinical path, in order to provide reliable estimates of effectiveness. Laboratories should be involved in assessment procedures to improve their capacity to adapt the offer of genetic testing according to evidence-based recommendations.

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The work was based on the valuable contribution of all laboratories participating to the survey.

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References


(33) Baglin T, Gray E, Greaves M et al. Clinical guidelines for
testing for heritable thrombophilia. Br J Haematol 2010; 149: 209-20


### 3.1 Introduction to the ACCE (Analytic validity, Clinical validity, Clinical utility, Ethical, legal and social aspects) model

Maria Rosaria Gualano, Benedetto Simone, Walter Ricciardi, Stefania Boccia

The acronym ACCE takes its name from the four principal domains of the evaluation process: A stands for Analytic validity, C for Clinical validity, C for Clinical utility and E for the associated Ethical, legal and social implications that may arise in the context of using the test (1).

This model is composed of 44 specific questions and, as shown in Figure 3.1, describes disorder, testing, and clinical scenarios, as well as the 4 main criteria mentioned above (2).

The **analytic validity** of a genetic test reflects how accurately and reliably the test measures the genotype of interest and it is focused on the laboratory component. There are four specific elements of analytic validity: analytic sensitivity or analytic detection rate, defines how effectively the test identifies specific mutations; analytic specificity, defines how effectively the test correctly classifies samples that do not have specific mutations; laboratory quality control, and assay robustness.

It is important to establish the difference between an assay, that is the technical measurement of a biomarker, and a test, that represents the application of that assay for a particular disease, in a particular population, for a particular purpose. A single assay can, therefore, be used in various different tests (3).

The **clinical validity** of a genetic test defines its ability to detect or predict the associated disorder (phenotype). The two Components of Clinical Validity to be assessed are: the scientific validity (the evaluation of the relationship between biomarker and disease) and the test performance (Evaluation of the test performance in the clinical situation) (3).

This dimension of the evaluation is comprised of six elements: clinical sensitivity (or the clinical detection rate), clinical specificity, prevalence of the specific disorder, positive and negative predictive values, penetrance, and modifiers (gene or environmental).

The clinical utility of a genetic test describes the elements that need to be taken into account when evaluating the risks and benefits associated with its introduction into clinical practice. Some other factors to be considered are: the availability and effectiveness of interventions aimed at avoiding adverse clinical consequences, the quality assurance assesses procedures in place for
controlling pre-analytic, analytic, and post-analytic factors, pilot trials assessing the performance of testing under real-world conditions and the economic evaluation, helpful to define financial costs and benefits of testing.

Additionally, *ethical, legal, and social implications* related to the utilization of a genetic test should be considered in the context of the other components. In fact, with expanding genomic innovations, ethical, legal and social implications become more complex: policy-makers need to become acquainted with genomics in order to implement adequate policies and rules.
3.2 Analytical validity of genetic tests for thromboembolism

Giovanni Ivaldi, Alberto Izzotti, Domenico Coviello

3.2.1 INTRODUCTION AND STUDY AIMS

Factor V Leiden (FVL; R506Q), the most common known inherited risk factor for thrombosis (4), results from a base change from G to A at position 1691 of the gene encoding coagulation Factor V. The associated amino acid substitution eliminates one of three activated Protein C cleavage sites in the Factor V protein, resulting in Factor V being inactivated more slowly and generating more thrombin, thereby enhancing the potential for clot formation. Prothrombin (Factor II) mutation (PT20210A) is the second most common known inherited risk factor for thrombosis (4). The 20210G_A gene variant produces an amino acid substitution in the PT protein, which results in higher circulating PT levels, to about 30% above normal in heterozygotes and to 70% above normal in homozygotes, with an enhanced potential for clot formation.

The accuracy of the laboratory performing the genetic testing is essential for providing a useful and safe service to clinicians and patients. The first parameter considered in the quality evaluation of a diagnostic laboratory is the analytical validity. As a general definition, analytic validity is a measure of the assay's performance, which includes its analytic sensitivity and specificity, and also its robustness.

Analytical sensitivity (or detection rate) is the proportion of affected individuals (or those who become affected within a specified period of time) with a positive (unfavorable) screening test result. It defines how effectively the test identifies specific mutations that are present in a sample (5).

Analytical specificity is the proportion of unaffected individuals with a negative screening test result. It defines how effectively the test correctly classifies samples that do not have specific mutations (polymorphisms/variants) (5).

Robustness refers to how resistant the test is to changes in preanalytic and analytic variables, like the source of the specimen or the temperature of the environment (5).

In the current clinical scenario, analytic validity is defined as a laboratory's ability to accurately and reliably detect the FVL mutation (R506Q) and a single PT mutation (20210 G_A). Given that there are two alleles for each of the genes, the genetic test(s) may identify individuals with no mutations, individuals with a single mutation (heterozygous), or individuals with two mutations (homozygous or compound heterozygous).

In our study, we report the results of genetic analysis in a population of 4,996 subjects during a 8 years follow up with the aim to verify the analytical validity in our sample populations and report on data present in the literature.

3.2.2 MATERIALS AND METHODS

DNA was extracted from blood lymphocytes with standard techniques in one laboratory at Galliera Hospital. Genetic polymorphism analyses for prothrombin II and V (Leiden Factor) were performed on 4,996 subjects testing one SNP for each gene (G20210A for prothrombin II; Arg506Gln for prothrombin V) by PCR and reverse dot blot technique (RDB) and/or by real-time PCR (RT-PCR). Direct sequencing of amplicons that include the region of interest has been used to verify the results of the other two techniques on a random selection of 200 samples. On a subset of 17 subjects one SNP was evaluated for prothrombin II (G20210A) and 3 SNPs (R506Q, H1299R, Y1702C) for prothrombin V by a different technology to analyze multiple genetic SNPs by microarray. Affymetrix 500 chip was used testing 500,000 SNPs approximately after Nsp or Sty enzymes restrictions according to the standard protocol (www.affymetrix.com).

3.2.3 RESULTS AND DISCUSSION

Genetic analyses for prothrombin V found 91% wild type subjects, 9% heterozygous subjects, and 0.2% homozygous mutants (11 subjects). Genetic analysis for prothrombin II found 94% wild type subjects, 6% heterozygous subjects, and 0.08% homozygous mutants (4 subjects).
Single SNP prothrombin II and V analyses indicate that 2 subjects were wild type for both genes, 9 subjects were heterozygous for one gene, 4 were heterozygous for both genes, 1 was homozygous mutant for prothrombin V, and 1 was homozygous mutant for both prothrombin II and V.

Multiple SNPs were analyzed in the subgroup of 17 subjects by microarray. The obtained response was 100% concordant with results obtained with the other techniques.

The conventional “gold standard” method for FVL and prothrombin G20210A detection, the bidirectional sequencing of the specific genetic region of the gene of interest, has shown 100% concordance with the other two techniques used: RDB and RT-PCR.

Two categories of studies are available to assess analytic validity: proficiency testing exercises and method comparisons between an experimental test and a referent test.

Proficiency testing programs assess laboratory performance by means of interlaboratory comparisons. The proficiency testing program sends blinded samples to multiple laboratories for testing. Our laboratory has participated in proficiency testing organized by European Molecular Quality Network (EMQN), part of a large consortium of diagnostic laboratories EUROGENTEST (www.erogentest.org). The EMQN organize several schemes for the proficiency testing (or External Quality Assessment-EQA), they send blinded samples to multiple laboratories for testing. The laboratory decide the methodology to use for the test, perform the analysis and the results are returned to EMQN. Participants typically receive reports from EMQN that describe the laboratory’s individual performance and the aggregate performance of the other participating laboratories. Proficiency testing programs document pre- and post-analytical testing errors and assay performance. They often categorize results obtained with specific assay methods and report consensus findings by participating laboratories of alleles tested and genotypes identified in each exercise. Data from proficiency testing have been suggested to be a reliable source for assessing overall laboratory performance under real-world conditions.

Our laboratory every year participate to the EQA and has obtained a score ranging between 85% and 95%, with maximum score for analytic sensitivity and specificity, but some comments on the wording of the written report.

On the subset of 17 subjects where the Affymetrix 500 chip was used, obtained data were tested for their quality by calculating the average response rate signals for each chip whose quality threshold is >90%.

In the very recent work published by The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (6) a review of the literature has been reported and referring to analytical validity. Collectively, these reports show an overall error rate of 1.0%, an analytic sensitivity of 98.8%, and an analytic specificity of 99.3%. They conclude that there is convincing evidence that analytic validity is high for both FVL and PT20210A and most laboratories can test for FVL and PT20210A with a high degree of reliability.

In a previos recent study (5), they report on forty-one studies where at least two methods were compared for FVL detection and for prothrombin G20210A. Analytical validity was evaluated and the concordance between the results obtained, ranged from 99.5% to 100%. In particular three studies addressed external quality assurance or laboratory performance. The first one has described the results of the United Kingdom National External Quality Assessment Scheme (UK NEQAS) Thrombophilia Screening Program (7). Two hundred eighty centers participated in the thrombophilia screening exercise. In the second one they have reported the results of The Royal College of Pathologists of Australasia’s external quality assurance program (8). That program sent 133 DNA samples with known mutations to laboratories in 10 separate surveys. For the 3 799 responses received, the overall successful identification rate was 98.6%. Finally, the third one (9) confirmed the findings described earlier. Their survey was organized by the Subcommittee on Hemostasis of the Italian Committee for Standardization of Laboratory Tests (CISMEL). They sent four samples with known genotypes to 52 participating laboratories and received 41 responses.

3.2.4 ANALYTIC VALIDITY CONCLUSIONS

Our results indicate that analytic validity is high for both FVL and PT20210A. Also the data reported in the literature show that most laboratories can test for FVL and PT20210A with a high degree of reliability.

Multiple SNPs analysis by microarray provides a remarkably wider piece of genetic information, which can be used as a better predictor for diseases occurrence.
The clinical validity of a genetic test defines its ability to detect or predict the associated disorder (phenotype). The two components of Clinical Validity to be assessed are: the scientific validity (the evaluation of the relationship between biomarker and disease) and the test performance (evaluation of the test performance in the clinical situation). This dimension of the evaluation is comprised of six elements: clinical sensitivity (or the clinical detection rate), clinical specificity, prevalence of the specific disorder, positive and negative predictive values, penetrance, and modifiers (gene or environmental).

### 3.3.1 SCIENTIFIC VALIDITY

Numerous individual studies, systematic reviews and meta-analyses have addressed the issue of inherited thrombophilia in terms of increased risk.

Gohil et al. (10) conducted a broader systematic and comprehensive meta-analysis on all candidate genes to assess their genetic contribution to the aetiology of venous thromboembolism (VTE) in all ethnic groups. Both provoked and unprovoked events were considered, including recurrences. Approximately 126 525 cases and 184 068 controls were considered from 173 case-control studies, which included 21 genes (28 polymorphisms). Statistically significant associations with VTE were identified for FVL (OR 9.45; 95% CI 6.72-13.30) and PT20210A (OR 3.17; 95% CI 2.19-3.46), in Caucasian populations. MTHFR resulted significantly associated with incidence of VTE in Chinese/Thai populations (OR 1.57; 95% CI 1.23-2.00). The study provided significant associations also for other, less frequent, genetic variants: factor V A4070G (OR 1.24; 95% CI 1.02-1.52), prothrombin G11991A, (OR 1.17; 95% CI 1.07-1.27), PAI-1 4G/5G, (OR 1.62; 95% CI 1.22-2.16) and alpha-fibrinogen Thr312Ala (OR 1.24; 95% CI 1.02-1.52, p = 0.008) in Caucasians and ACE I/D in African American populations (OR 1.5; 95% CI 1.03-2.18, p = 0.03).

In order to assess the risk of first unprovoked VTE events related to FV, PT20210A and MTHFR variants, we performed an individual patient data (IPD) of 36 studies (11). 10 000 adult patient cases and 20 000 controls were pooled, with analysis being adjusted by age and gender. All the individuals considered for the analysis were at least 16 years old and experiencing their first thromboembolic event. Oncologic patients and patients affected by other conditions that are known risk factors for thromboembolic events (inflammatory bowel diseases, autoimmune conditions, Beçet disease, transplant) were excluded. Based on literature and on biologic plausibility, the effect of FVL and PT20210A was analysed applying a dominant model (carriers of the variant Vs non-carriers). For MTHFR a recessive model was used [homozygous carriers Vs (heterozygous carriers and non carriers)]. FVL resulted associated with the highest risk of developing VTE (overall OR: 3.51, CI: 2.53-4.87). The risk was higher in younger individuals (<45 years old, OR: 4.26, CI: 2.67–6.81; ≥45 years old, OR: 2.88, CI: 2.09–3.97) and in men (male population, OR: 3.87, CI: 2.48-6.03; female population, OR 3.22, CI: 2.31–4.50). As expected, women assuming oral contraceptives (OCs) resulted as the population with the highest risk of developing VTE (women assuming OCs, OR: 5.63, CI: 3.26 – 9.75; women not assuming OCs, OR: 2.93, CI: 1.88–4.56). The stratifications showed a higher risk for FVL carriers to develop venous thrombosis without embolism (OR: 4.49, CI: 3.23 – 6.24) and for cerebral venous sinus thrombosis (OR: 4.14, CI: 2.46–6.97).

PT20210A resulted associated with an increased risk of developing VTE (overall OR 2.48, CI: 1.86-3.29), albeit not as strongly as FVL (Table 3.1). The stratifications did not show great differences in the risk for specific categories. Again, younger carriers of the polymorphic variant resulted at higher risk than their older counterparts (< 45 years old, OR: 2.65, CI: 1.84–3.83; ≥ 45 years old, OR: 2.17, CI: 1.52–3.09). The risks for men (OR: 2.20, CI: 1.59–3.05) and women were similar (OR: 2.49, CI: 1.82–3.42), and the assumption of OCs affected the risk of developing VTE (women not assuming OCs, OR: 2.10, CI: 1.21–3.65; women assuming OCs, OR: 3.96, CI: 2.43–6.45). The stratification by outcome showed a particularly strong association of the polymorphic variant with regard to the risk
### TABLE 3.1

**RISK OF DEVELOPING VTE IN PRESENCE OF FVII, PT20210A AND MTHFR, OVERALL AND STRATIFIED BY AGE GROUPS, GENDER, ASSUMPTION OF ORAL CONTRACEPTIVES (OC) AND TYPE OF THROMBOEMBOLIC EVENT**

<table>
<thead>
<tr>
<th>Case/Controls</th>
<th>Cases/Controls</th>
<th>OR *</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Not Exposed</td>
<td></td>
</tr>
<tr>
<td>Overall (Dominant model)</td>
<td>1441/1059</td>
<td>9104/20490</td>
<td>3.51 2.53-4.87</td>
</tr>
<tr>
<td>Younger than 45</td>
<td>801/370</td>
<td>4480/6946</td>
<td>4.26 2.67-6.81</td>
</tr>
<tr>
<td>45 and older</td>
<td>633/789</td>
<td>4528/13484</td>
<td>2.88 2.09-3.97</td>
</tr>
<tr>
<td>Women</td>
<td>840/638</td>
<td>5497/11167</td>
<td>3.22 2.31-4.50</td>
</tr>
<tr>
<td>- Not assuming OC</td>
<td>137/473</td>
<td>2395/7426</td>
<td>2.93 1.88-4.56</td>
</tr>
<tr>
<td>- Assuming OC</td>
<td>217/61</td>
<td>1184/1329</td>
<td>5.63 3.26-9.75</td>
</tr>
<tr>
<td>Men</td>
<td>594/519</td>
<td>3511/8752</td>
<td>3.87 2.48-6.03</td>
</tr>
<tr>
<td>VT</td>
<td>855/1063</td>
<td>3396/14498</td>
<td>4.49 3.23-6.24</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>409/1048</td>
<td>1959/14756</td>
<td>3.46 2.71-4.42</td>
</tr>
<tr>
<td>CVT</td>
<td>53/101</td>
<td>291/2394</td>
<td>4.14 2.46-6.97</td>
</tr>
<tr>
<td>Splanchnic thrombosis</td>
<td>8/62</td>
<td>200/1977</td>
<td>1.30 0.61-2.79</td>
</tr>
</tbody>
</table>

| Overall (Dominant model)          | 732/512        | 9813/21137 | 2.48 1.86-3.29 |
| Younger than 45                    | 416/229        | 4865/7087  | 2.65 1.84-3.83 |
| 45 and older                      | 312/283        | 4849/33990 | 2.17 1.52-3.09 |
| Women                             | 440/296        | 5897/11509 | 2.49 1.82-3.42 |
| - Not assuming OC                  | 142/188        | 2570/7711  | 2.10 1.21-3.65 |
| - Assuming OC                      | 138/35         | 1263/3155  | 3.96 2.43-6.45 |
| Men                               | 288/215        | 3817/9056  | 2.20 1.59-3.05 |
| VT                                | 510/754        | 3798/14900 | 2.60 1.94-3.47 |
| Thromboembolism                   | 250/758        | 2085/14894 | 3.00 2.30-3.90 |
| CVT                               | 51/100         | 303/2723   | 4.40 2.18-8.91 |
| Splanchnic                        | 17/78          | 192/1969   | 2.10 1.17-3.77 |

| Overall (Recessive model)         | 418/1213       | 10127/20436 | 1.26 0.86-1.86 |
| Younger than 45                    | 169/381        | 5112/6935  | 1.45 0.98-2.16 |
| 45 and older                      | 227/821        | 4934/13452 | 1.69 0.95-3.01 |
| Women                             | 217/670        | 6120/11135 | 1.12 0.80-1.59 |
| - Not assuming OC                  | 64/436         | 2648/7463  | 0.90 0.74-1.10 |
| - Assuming OC                      | 13/62          | 1388/1328  | 0.67 0.42-1.06 |
| Men                               | 179/521        | 3926/8750  | 2.23 1.22-4.07 |
| VT                                | 163/1021       | 789/3637   | 1.33 1.03-1.72 |
| Thromboembolism                   | 116/1033       | 891/9453   | 1.15 0.82-1.62 |
| CVT                               | 22/130         | 164/888    | 1.34 0.64-2.82 |
| Splanchnic                        | 5/63           | 13/252     | 1.92 0.50-7.45 |
of developing a thrombosis of the cerebral venous sinus (OR: 4.52, CI: 2.89–7.06).

As for MTHFR, the overall analysis (OR: 1.26; CI: 0.86–1.86) and the stratified analyses indicated that the MTHFR variant is not associated with a significantly increased risk of developing a VTE. Men, however, resulted at risk of developing VTE in presence of the polymorphic variant (OR: 1.84; CI: 1.24–2.74).

Women assuming OCs resulted the category at the highest risk (OR: 2.05; CI: 1.1–3.79). No type of cardiovascular accident considered resulted significantly associated to the presence of the polymorphic variant.

Carriers of both FVL and PT20210A have an OR of 3.22 (CI: 1.07–9.68). Notably, women assuming oral contraceptives have a very increased risk of VTE (OR: 23.47, CI: 4.58–120.23). The stratification by outcome showed particularly increased risks for individuals with both FVL and PT20210A variants to develop a cerebral venous sinus thrombosis (OR: 7.38, CI: 0.99–55.06).

### 3.3.2 TEST PERFORMANCE

#### Clinical sensitivity, detection rate

Overall, the clinical sensitivity of the factor V Leiden mutation is between 20% and 50%, and that of PT20210A varies between 5% and 19% (12).

The CDC has produced several reports on genetic tests. As for inherited thrombophilias, the evaluations focused solely on recurrent events: FVL clinical test sensitivity is 28%, with a 95% CI of 12.9-34.6%, whereas the overall clinical sensitivity of PT20210A is 11%, with a 95% CI of 6.2-21.1% (13).

#### Clinical specificity

Low penetrance of FVL or PT20210A is the main reason why clinical specificity is less than 100%. Analytic error is possible, but likely to be a much smaller factor in clinical false positive test results.

Clinical specificity for the FVL and PT20210A tests have not been firmly established, but the overall FVL specificity of VTE for recurrences is 81%, with 95% confidence intervals of 73.3-95.9%. Specificity for PT20210A is 81%, with 95% CI of 93.2-94.4% (13).

#### Prevalence of the disorder

As of today, the most comprehensive epidemiological data on the prevalence and
incidence of VTE have been generated from studies of specific populations. Studies from specific geographical areas in the US, which included patients with only incident VTE at home or in hospital, revealed a VTE incidence of 71–117 per 100,000 person-years (14-15). Approximately 450,000 cases of deep venous thrombosis, 350,000 cases of nonfatal pulmonary embolism, and 250,000 cases of fatal pulmonary embolism are estimated to occur annually in the United States overall (16-18).

The most comprehensive data from Europe refer to studies in France (19) and Sweden (20) that included both incident and recurrent VTE events, reporting an overall incidence of 160–180 per 100,000 person-years. An epidemiological model estimated the yearly incidence of VTEs in six of the most populous countries in Europe (France, UK, Italy, Germany, Sweden and Spain) close to 500,000 (21).

A detailed estimate of the prevalence of VTE events, however, is hard to obtain because VTE is difficult to diagnose due to several factors: VTE is often clinically silent and, in many cases, the first sign of the disease is a sudden fatal PE (22-23). Despite modest increases in antemortem diagnosis of PE over the years, less than half of autopsy detected PE cases are diagnosed antemortem (24). The lack of postmortem examinations performed on a routinely base causes an underestimation of their incidence. Only 29% of patients who survive an initial embolic event are diagnosed with VTE (20). However, asymptomatic DVT is strongly associated with the development of symptomatic VTE (25-27) and with an increased risk of death (21, 28).

Incident venous thromboembolism is triggered by a confluence of situational (e.g., trauma, surgery, cancer) and genetic risk factors. The two most common genetic contributors are the Factor V Leiden mutation (FVL) and the prothrombin (PT) 20210A mutation. Methylene tetrahydrofolate reductase (MTHFR) C677T has been shown not to be associated with increased risk of VTE although it is, with FVL and PT20210A, the most common gene investigated in the past decades as putative risk factors for VTE (11).

Penetrance

Studies on penetrance of FVL show that heterozygous FVL individuals have a 10% lifetime risk of developing a VTE, while homozygous present a risk of about 80% (29).

Penetrance is heterogeneous and age-related. FVL often develop the first VTE episode after 45 years of age, especially in association with circumstantial risk factors (trauma, surgery, pregnancy, oral contraceptives) or even minor events (long journey, minor surgery) (30-34). About 50% of FV Leiden carriers have a VTE episode by the age of 65 years, whereas the lifetime risk of thrombosis of asymptomatic relatives with FV Leiden is not higher than 25% (32-34).

The scientific evidence is not equally exhaustive on PT20210A. The risk of thrombosis in PT20210A heterozygotes is estimated to be 2-4 fold higher than those wild type homozygotes and further increased for homozygotes (35).

Because of its high prevalence in the general population, the homozygous C677T polymorphism of the MTHFR gene is often detected and considered erroneously as a thrombophilic defect, but it is not associated with an increased risk of developing VTE.
3.4 Clinical predictivity of genetic tests for thromboembolism

Alberto Izzotti, Emilio Di Maria, Claudia Casella, Giovanni Ivaldi, Domenico Coviello, Marina Vercelli

3.4.1 INTRODUCTION AND STUDY AIMS

Although genetic tests for evaluating thrombotic risk as related to endogenous risk factors are commonly used, the evaluation of their clinical predictivity is still an open question. Several studies have associated an increased risk for the occurrence of thromboembolism with the existence of adverse genetic polymorphisms especially for prothrombin II and V (Leiden factor) (36). One of the major problems limiting the clinical predictivity of these genetic tests is the scanty information provided as compared to the real genomic situation. Genetic risk in non-monogenic chronic-degenerative diseases, such as atherosclerosis and cancer, is a complex trait arising from the interaction of multiple adverse genetic assets with environmental exposures (37). Accordingly, it is unlikely that a single genetic test may bear remarkable clinical predictivity for chronic-degenerative diseases, which are typical multi-factorial diseases arising from the interaction of multiple risk factors of both exogenous and endogenous origin. A further issue limiting the clinical predictivity of genetic test for degenerative diseases is that nowadays they analyse only one or very few single nucleotides polymorphism (SNP) of the explored gene despite the established existence of multiple polymorphisms. Thus, the genetic information provided by analyzing one single SNP for prothrombin genes is limited and cannot be directly depict the real risk for developing a complex multifactorial diseases such as vascular thromboembolism. In order to shed light on this still open issue, we performed the herein presented study aimed at comparing the clinical predictivity of genetic test as currently performed by analyzing single genes for single SNP by qPCR with those of genetic tests performed by analyzing multiple genes for multiple SNPs by using a microarray based approach. Two parallel studies were performed.

The first study was a retrospective analysis on a cohort of 4,996 undergoing genetic tests for prothrombin II and V. For these subjects, the history of hospital admission during the 2000-2007 period was reconstructed. The aim of this study was to evaluate the clinical predictivity of monogenic test analyzing single SNP in the population of the Ligurian region in Italy.

The second study was performed on 17 subjects randomly selected from the first cohort. Inclusion criteria were the availability of single gene tests (for both prothrombin II and V), of clinical history, and of good quality frozen DNA samples. These subjects underwent multiple gene and SNPs analysis by microarray focusing on genes pathogenically relevant for thromboembolism. The aim of this study was to establish whether or not multiple gene testing is more predictive than single gene test for the clinical outcome, i.e. hospital admissions related to thromboembolic event.

3.4.2 STUDY 1. ANALYSIS OF THE CLINICAL PREDICTIVITY OF SINGLE GENE TESTS BY EPIDEMIOLOGIC RETROSPECTIVE STUDY

3.4.2.1 Materials and Methods

Genetic polymorphism analyses for prothrombin II and V (Leiden Factor) were performed on 4,996 subjects testing one SNP for each gene (G20210A for prothrombin II; Arg5406Gln for prothrombin V) by qPCR. Single subject hits, identified by referring to the fiscal code and birth date, were cross-linked with the Regional Ligurian Registry of Pathology recording hospital admission during the 2000-2007 period (8 years). Thus it was possible to identify those subjects analyzed for genetic polymorphisms undergoing hospital admission as well as the hospitalization cause. The relationship between gene polymorphism and diseases occurrence was tested by logistic regression analysis.
### Results and Discussion

The average age of the examined population was 53.5 ± 18.8 years, 65% females and 35% males. Genetic analyses for prothrombin V found 91% wild type subjects, 9% heterozygous subjects, and 0.2% homozygous mutants (11 subjects). Genetic analysis for prothrombin II found 94% wild type subjects, 6% heterozygous subjects, and 0.08% homozygous mutants (4 subjects).

Out of a total of 4,996 subjects, 1,385 underwent hospital admission during the examined period having a hospitalization cause amenable to the occurrence of thromboembolic events. Hospitalizations causes with their frequencies in percentages are reported in Figure 3.2.

The frequencies of diseases occurrence as related to genetic polymorphism for prothrombin V was 41% in wild-type and 39% in heterozygous carriers, and 45% in homozygous carriers with no significant difference. The difference between heterozygous carriers and wild-type/heterozygous was not statistically significant (P=0.3376). It should be noted that this result was obtained by analyzing 11 homozygous subjects only bearing homozygous mutation and only 5 undergoing hospital admission. Similarly, diseases occurrence as related to genetic polymorphism for prothrombin II was 40% in wild-type, 41% in heterozygous carriers, and 50% in homozygous carriers. The difference between homozygous carriers and wild-type/heterozygous was statistically significant (P=0.038). However it should be noted that this result was obtained by analyzing 4 homozygous subjects only both bearing homozygous mutation and only 2 undergoing hospital admission.

A strong relationship between age (X) and the number of thromboembolic event (Y) was detected in the overall population with the best fit obtained by linear regression (Y = -0.325 + 0.027 * X, r=+0.304, P<0.0001). This finding indicates that no age threshold exists for the risk of thromboembolic event and accordingly primary prevention should not be performed only in the elderly but also in young subjects. However, the genotype asset influences the risk of thromboembolic event at different ages. Indeed, as reported in Table 3.2, the OR for thromboembolic event as related to adverse genotype asset is higher at younger ages and lower at older ages. This findings indicate that endogenous factors are relevant for defining risk disease at younger ages when environmental and lifestyle factors exposures has not yet lasted enough to induce clinically relevant adverse effects. Conversely, at older ages, environmental and lifestyle factors exposure lasting for decades prevails on endogenous genetic factors in determining disease risk. These results are in agreement with the matter of fact that duration exposure is a major factor affecting the adverse effects of environmental exposures (38). Logistic regression analysis indicated that male gender (OR 2.1, CI 1.09-2.95) and age >52 years (OR 5.3, CI 2.70-10.57) and age >74 (OR 9.1, CI 4.48-18.45) were the main risk factors affecting the probability of undergoing hospitalization for thromboembolic events.
3.4.3.1 Materials and Methods

On the 17 subjects one SNP was evaluated for prothrombin II (G20210A) and 3 SNPs (R506Q, H1299R, Y1702C) for prothrombin V by qPCR using DNA extracted from blood lymphocytes. The same samples were used to analyze multiple genetic SNPs by microarray. Affymetrix 500 chip was used testing 500,000 SNPs approximately after Nsp or Sty enzymes restrictions according to the standard protocol (www.affymetrix.com). Obtained data were tested for they quality by calculating the average response rate signals for each chip whose quality threshold is >90%. Data related to SNPs of genes pathogenically involved in thromboembolism were coded as wild type, heterozygous, homozigous mutant and analyzed for their clinical predictivity by hierarchical cluster analysis and k-nearest neighbors algorithm using Genespring software (Agilent, CA, USA).

3.4.3.2 Results and Discussion

Single SNP prothrombin II and V qPCR analyses indicate that 2 subjects were wild type for both genes, 9 subjects were heterozygous for one gene, 4 were heterozygous for both genes, 1 was homozygous mutant for prothrombin V, and 1 was homozygous mutant for both prothrombin II and V. Among these subjects 2 underwent hospitalization admission related to thromboembolism during the monitored period (2000-2007). One of these subjects was heterozygous for prothrombin V only, the other was heterozygous for both prothrombin II and V. No hospital admission was recorded for other subjects bearing double heterozygosis or mutated homozygosis for one or both genes. Accordingly, single SNP analysis for prothrombin II and V was not predictive of the clinical outcome in the examined cohort. These findings are in agreement with previous studies performed in atherosclerotic patients reporting that prothrombin II and V SNPs are significantly related with diseases onset only in case of homozygous mutant exposed to cigarette smoke (39). Indeed, it is conceivable that the genetic risk factors mainly exert its action as modulator of exposures effect with a typical gene-environment interaction that is the real risk factor for chronic-degenerative diseases (38).

Multiple SNPs were analyzed in the same 17 subjects by microarray. Obtained response call was satisfactory (94.3±0.02%, mean ± SD). The list of genes involved in thromboembolism as well as the number of their SNPs spotted on the microarray is reported in Figure 3.11 genes were examined accounting for a total of 197 thromboembolism-related SNPs. By comparison, qPCR performed on the same samples analyzed only 2 genes accounting for a total of 4 SNPs.

The whole SNPs profile of these 197 SNPs genes was used as biomarker evaluating its predictivity for diseases onset. As depicted in the hierarchical cluster analysis reported in Figure 3.4, the 15 subjects devoid of clinical events were characterized by a similar SNPs profile located on the right part of the hierarchical tree. Conversely, the 2 subjects undergoing hospitalization due to clinical events amenable to thromboembolism were characterized by a different SNPs profile.
located in the right part of the hierarchical tree. The k-nearest neighbors’ algorithm was used to predict the diseases status basing on SNPs profile. This bioinformatic tool correctly classified all the 17 samples for their disease status with 17 correct predictions, 0 incorrect prediction, 0 unpredicted. These results indicate that multiple SNPs analysis is more predictive of the diseases status than single SNP analysis.

**Gene SNP**

**N° SNPs**

- FV: 33
- FIII: 5
- MTHFR: 4
- SERPIN C1 (ANTITHROMBIN III): 7
- PROS1 PROTEIN S: 5
- PROCR (PROTEIN C ENDOTHELIAL RECEPTOR): 1
- ITGB3 (GLYCOPROTEIN IIIA): 24
- ITGB2 (GLYCOPROTEIN IIA): 26
- FXII_B: 5
- FXII_A: 84
- FIBRINOGEN: 3

**Fig. 3.4**

**Hierarchical Cluster Analysis Relating the Analysis of 197 SNPs by Microarray with the Clinical Outcome**

Each column represents one subject for a total of 17 examined samples. Each horizontal line represents one SNPs colored according to its polymorphism (blue wild type, yellow heterozygous mutant, red homozygous mutant). Patients are clustered together in the upper hierarchical tree depending on the similarity of their SNPs profile. For each column colored bottom square indicates the disease status (yellow no hospitalization; red occurrence of thromboembolism-related hospitalization events). The two subjects having clinical outcomes (right part of the hierarchical tree) are characterized by a SNPs profile different from those of the other 15 subjects (left part of the hierarchical tree).
3.4.4 CONCLUSIONS

In conclusion, presented results provide evidence that the analysis of single SNPs for prothrombin II and V may be predictive for thromboembolism occurrence only for homozygous mutant, which are very rare in the population, and mainly at younger (<54 years) age. At older ages environmental exposures and lifestyle factors overwhelm the role of genetic risk factors. Single SNP analysis as currently performed in clinical practice makes available only a very small piece of information as compared to the whole individual genetic asset. Multiple SNPs analysis by microarray provides a remarkably wider piece of genetic information, which can be used as a better predictor for diseases occurrence.
CHAPTER 3: CLINICAL UTILITY

3.5 Clinical Utility

Corrado De Vito, Lamberto Manzoli, Elvira D’Andrea, Anna Miani, Carolina Marzuillo, Antonio Boccia, Paolo Villari

3.5.1 INTRODUCTION

Clinical utility, in the context of the framework developed by the U.S. Task Force on Genetic Testing is considered as the balance of benefits to risks, and thus, the Task Force recommended: “before a genetic test can be generally accepted in clinical practice, data must be collected to demonstrate the benefits and risks that accrue from both positive and negative results” (40). Originally, the Task Force contemplated as an aspect of the clinical utility also the assessment of the social and psychological benefits or harms of the genetic information, or, in other words, the ethical, legal and social implications of the genetic tests. However, this interpretation of the clinical utility was not accepted unanimously and successively it was proposed to list the psychosocial outcomes of testing in a different category called ELSI (Ethical, Legal and Social Implications) (41). The concept of clinical utility was subsequently developed by the major framework for the evaluation of genetic tests: the ACCE model. In this framework the clinical utility focuses specifically on the health outcomes (both positive and negative) associated with testing, taking into account the natural history of the clinical disorder and the availability and the effectiveness of interventions aimed at avoiding adverse clinical consequences (if no effective interventions are available, for example, testing may not be warranted) (42). A critical question to be answered before the introduction of a new DNA test is if there is an effective remedy, an acceptable action, or other measurable benefit. If the disorder of interest cannot be either treated or avoided, it is unlikely that justification can be made for routinely identifying it. Having an effective intervention to prevent or avoid the morbidity or mortality associated with the disorder (including risk-reducing behavior) is essential to address the decisions about the use of a test for population screening.

The standard framework of the ACCE model was used to assess the clinical utility of predictive genetic testing for venous thromboembolism (VTE) (Factor V Leiden, G20210A and MTHFR). Thus the natural history of the clinical disorder was analyzed and the impact of the results on the treatment and the effective preventive intervention in case of positive test was assessed. To fulfill these aims a systematic review and a quality assessment of the existing clinical guidelines about prevention and treatment of VTE in case of positive test was performed. Finally, we reported the assessment of the clinical utility of genetic testing predictive of VTE in the specific population of women taking oral contraceptives (OC).

3.5.2 RISK FACTORS AND CLINICAL EVOLUTION OF VTE

Pregnancy and oral contraceptive (OC) use are also recognized factors associated to VTE (for other risk factors see Chapter 1). The incidence of pregnancy-associated venous thromboembolism has been estimated to be one or two per 1 000 pregnancies (43). Pulmonary embolism occurs in approximately 16% of patients with untreated deep-vein thrombosis (DVT), and is the most common cause of maternal death (44). Also association between oral contraceptive (OC) use and VTE has long been recognized (45). The only meta-analysis comparing the risk of VTE in OC users versus non-users, published in 1995, found that the use of oral contraceptives is associated with a three-fold increase in VTE risk (46). Successively, other two meta-analyses showed that third generation OCs increase VTE risk more than second generation preparations (47, 48).

The extent of the health burden attributed to VTE in terms of the total number of incident and recurrent non-fatal DVT and PE clinical events, and VTE-related deaths per year has been calculated in six EU countries (France, Germany, Italy, Spain, Sweden, UK) (49). The results of the study indicate that VTE is a major public-health problem in these countries, with a predicted total number of DVT events just under half a million and almost a third of a million PE events per year. Furthermore, a third of a million deaths occur per year due to sudden PE or following undiagnosed and untreated VTE.

The major complications of venous thromboembolism are the post-thrombotic syndrome, which can manifest as venous ulcer, and the chronic thromboembolic pulmonary
hypertension (CTEPH). The post-thrombotic syndrome is a result of the venous hypertension due to outflow obstruction and damage to the venous valves, and it develops in 20-50% of subjects (50), even when optimal anticoagulant therapy is used to treat DVT. Clinical characteristics are leg pain, skin changes and swelling. The incidence of the CTEPH is difficult to assess and it is actually underestimated. Early autopsy studies showed a prevalence of CTEPH of about 0.1-0.5%, while recent longitudinal studies indicate an incidence of approximately 4% (51). Physicians need to be more aware of complications of VTE, even in patients with no clinically obvious symptoms.

3.5.3 BIBLIOGRAPHIC SEARCH OF THE EXISTING GUIDELINES

The existing guidelines concerning health interventions to reduce morbidity of VTE in subjects with genetic risk of thromboembolism has been identified through a systematic search of scientific electronic databases such as MEDLINE and EMBASE and through a hand search of the retrieved literature. Web sites of all main national and international agencies and medical specialty societies involved in the production of guidelines were explored. Practice guidelines were included if the following inclusion criteria were met: a) the guidelines must contain systematically developed recommendations, strategies or other information to assist health care decision making in specific circumstances; b) the guidelines must have been produced under the auspices of a relevant professional organization; c) the guideline development process must have included a verifiable, systematic literature search and review of existing evidence; d) the guideline must have been developed or revised within the last 7 years.

The quality evaluation of the guidelines on genetic tests has been performed using the quality assessment tool developed by AGREE (Appraisal of Guidelines Research and Evaluation), a checklist proposed by a European collaboration aimed at developing a common instrument for the quality assessment of guidelines of medical practice (52). AGREE consists of 23 key items organised in six domains. Each domain is intended to capture a separate dimension of guideline quality: scope and purpose; stakeholder involvement; rigour of development; clarity and presentation; applicability; editorial independence. Each item is rated on a four-point scale and a following overall assessment is provided by the appraiser on a four-point scale indicating the grade of recommendation from “unsure” to “strongly recommended”.

Of the fourteen guidelines retrieved, four were excluded because were not produced under the auspices of a relevant professional organization. Further two guidelines were excluded after a closer analysis because did not report strategies or other information to assist health care decision making in specific circumstances. Eight guidelines fulfilled the inclusion criteria (Table 3.3) and have consequently been included in the study (53-60). Three of these are from USA (55, 56, 59), two from UK (53, 60), one from Australia (57), one under the auspices of WHO (58) and one from the association of the most important European foundations in the field of thrombophilia (54). All the included guidelines have been produced or updated from 2003 to 2010. Five guidelines are strongly recommended (55, 57-60), according to the grading of the AGREE system. Three guidelines are recommended with provisos or alteration (53, 54, 56), in particular because of the absence of criteria for including or excluding evidence identified by the search and the absence of description of the methods used to formulate the recommendations; the lack of externally review before the publication; the absence of a described procedure for updating the guideline; and finally because the potential organisational barriers in applying the recommendations have not been discussed.

The retrieved guidelines (Figure 3.5), particularly those more recent and of higher methodological quality, were used to get the evidence on the effectiveness and the safety of available interventions in case of a positivity to a genetic predictive test for VTE. Original primary studies and important meta-analyses were also taken into consideration, although they were not systematically searched and reviewed.

3.5.4 IMPACT OF POSITIVE OR NEGATIVE TEST ON PATIENT TREATMENT AND EFFECTIVE PREVENTIVE INTERVENTIONS IN CASE OF POSITIVE TEST

The objective of the evaluation of clinical utility in the specific context of predictive genetic testing for venous thromboembolism is to evaluate, on the base of the scientific evidence, whether there is an effective remedy, an acceptable medical intervention, or other measurable benefit in the
event of a positive test. If the disease in question cannot be treated or prevented, it is very unlikely that use of routine testing is justified. Having an effective intervention to prevent or avoid the morbidity or mortality (including behavioural changes involving a reduction in risk) is the crucial point to decide how to use a genetic test for the screening of populations or groups of individuals.

The clinical utility can be investigated in four levels that describe the objectives of the questions provided by the ACCE model: 1) the diagnostic thinking, which is the value of information in relation to diagnosis and prognosis; 2) the choice of therapy, namely the use of test results in the clinical management of the patient; 3) the assessment of patient outcomes that is the impact on survival or quality of life of the subject; 4) the social impact, including the cost-effectiveness analysis (41). Even if each of these points can influence the ultimate impact of using the test in clinical practice, however, from the clinical perspective, diagnostic thinking and therapeutic choice may constitute the basis of clinical utility, even in absence of data on health outcomes or cost-effectiveness.

In the collection of clinical recommendations evaluated (Table 3.3), there is no indication to a primary preventive approach for subjects with positive genetic tests predictive of VTE without a positive clinical history for VTE or other risk factors. In this situation, the knowledge of the information given by the test result does not change from a prognostic point of view because there is no prophylaxis and treatment protocol to follow in case of positivity. By contrast, the value of information changes in presence of intercurrent events which might endanger the health and survival of the subjects with a positive genetic test result.

According to the guidelines evaluated, intercurrent events play an important role in increasing the risk in patients with an established presence of genetic thrombophilic mutations. These events include: recurrent VTE, pregnancy, use of oral contraceptives, surgery and travels that provide a period of prolonged immobilization (> 8h). In these cases, the recommendations of the guidelines are indications for prophylaxis with a preventive treatment.

We talk about recurrent VTE when there is a new confirmed venous thrombotic complication after a first episode of VTE. One-third of patients develop a new event of thromboembolism within about 8 years after a first episode (61), and some authors described an increased risk of recurrent VTE due to a genetic thrombophilic mutation (62). As reported in several studies, the homozygous and the double heterozygotes carriers for factor V Leiden, prothrombin G20210A and MTHFR mutations, have a stronger association to the risk than...
heterozygotes (63, 64); however, the choice of the therapy recommended in all patients with genetic thrombophilic mutations, regardless of the strength of association, is the same. The prophylaxis recommended by European and American guidelines is to assume anticoagulants for an extended period of time, at least 6 months (long-term treatment), or a lifelong treatment with a vitamin K antagonist in selected patients with added risk factors, although the decision to undertake this type of therapeutic approach is controversial. As a matter of fact, this long-term decision should be based on balancing the long-term mortality risk from recurrent VTE, largely preventable with oral anticoagulant therapy, against the long-term mortality risk of major bleeding, the most frequent complication of oral anticoagulant therapy (65). Dose and duration of the treatment are unaffected by the carrier status, and are based, as with non-carriers, on the determination of standard parameters commonly used (specifically the International Normalised Ratio, a derived measure of prothrombin time) (54, 66).

As already stated, the association between venous thromboembolism and pregnancy in women with genetic mutations has been well documented and about 60% of cases of gestational thromboembolism is associated with the state of carriers of genetic thrombophilic mutations (56). According to several studies, women with Factor V Leiden or Factor G20210A homozygosis, or combined heterozygosis for Factor V Leiden and Factor G20210A, are

<table>
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<th>Title</th>
<th>Nation</th>
<th>Year</th>
<th>Agree Evaluation</th>
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<tr>
<td>British Thoracic Society (53)</td>
<td>British Thoracic Society guidelines for the management of suspended acute pulmonary embolism</td>
<td>UK</td>
<td>2003</td>
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<td>USA</td>
<td>2009</td>
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<td>National Health and Medical Research Council (57)</td>
<td>Clinical Practice Guideline for the Prevention of Venous Thromboembolism in Patients Admitted to Australian Hospitals</td>
<td>Australia</td>
<td>2009</td>
<td>Strongly recommended</td>
</tr>
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<td>2009</td>
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<td>2010</td>
<td>Strongly recommended</td>
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<tr>
<td>Scottish Intercollegiate Guidelines Network (60)</td>
<td>Prevention and management of venous thromboembolism. A national clinical guideline</td>
<td>UK</td>
<td>2010</td>
<td>Strongly recommended</td>
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considered to be at high risk and should be treated more aggressively compared to women with heterozygous Factor V Leiden or Factor G20210A mutations that are considered to be at moderate risk (54). In both cases prophylaxis could be recommended: first of all, women bearing genetic factors that increase the risk of VTE must be informed about the correlation of pregnancy with the potential development of VTE to promptly implement a program of prophylaxis. During pregnancy, prophylaxis should be implemented with low molecular weight heparin at prophylactic (4 000-5 000 U/die), intermediate (10 000 U/die) or adjusted dose (weight-adjusted, 200 U/kg) for high-risk groups (Factor V Leiden or Factor G20210A homozygosis, or combined heterozygosis for Factor V Leiden and Factor G20210A) regardless of the presence of a positive clinical history for VTE. For women with moderate risk (Factor V Leiden or Factor G20210A heterozygous) prophylaxis with heparin at prophylactic dose would be appropriate if they have more risk factors such as family history of VTE, age, immobilization, etc. or if they experienced previous episodes of VTE. Immediately after pregnancy, for both groups (high risk and moderate risk), prophylaxis should be continued with low molecular weight heparin or oral anticoagulants together with the use of elastic stockings for 6 weeks is recommended (67). In case of history of VTE, the recommendations include also a duplex ultrasound scan to serve as a reference and the use of elastic stockings. The women under long-term or lifelong therapy for VTE, during the pregnancy, must shift from oral anticoagulants, because of their teratogenic effects, to subcutaneous injections of low molecular weight heparin, resuming anticoagulants only at the end of pregnancy (68).

The use of OC predispose to the increase in risk of VTE, according to the generation of OC used, the oestrogen dose and the formulation of the compound. Despite the high level of the relative risk, the absolute risk is low and, also in relation to cost-effectiveness of testing, the guidelines drawn up by the WHO do not recommend mass screening before the first prescription (58). There are no recommendations that deny the prescription of oral contraceptives in women with thrombophilic mutations although the use in these cases is discouraged.

The role of the prophylaxis in case of surgical interventions has been addressed in the 8th edition of the ACCP Guidelines (55) and in the guidelines produced by the Institute for Clinical Systems Improvement (59). Patients with high risk (homozygosis and double heterozygosis for Factor V Leiden and Factor G20210A) are strongly advised to carry on an anticoagulant therapy with therapeutic doses of low molecular weight heparin or subcutaneous heparin. The recommendation for patients with heterozygous state (moderate risk group) is to begin treatment with prophylactic dose of low molecular weight heparin.

Owning genetic thrombophilic mutations is one of the risk factors for the development of VTE during long distance travels, with the increased risk persisting for about 8 weeks after the travel. Given the conflicting views about the use of thromboprophylaxis in travellers, there is insufficient evidence to support the routine use of active thromboprophylaxis measures in any group of travelers. However, it is reasonable to advise passengers to reduce venous stasis and to avoid dehydration, although these measures have also not been assessed in clinical trials (69).

3.5.5 RESULTS OF A SYSTEMATIC REVIEW AND META-ANALYSIS TO EVALUATE THE CLINICAL UTILITY OF THREE GENETIC TESTS FOR VTE IN WOMEN ASSUMING ORAL CONTRACEPTIVES

Given the large number of formulations, dosages and characteristics of studies, a meta-analysis was carried out to summarize the existing evidence on the association between VTE and OC use and to investigate how such association may vary according to several OC, users and study characteristics. The final goal of the study was to find the formulations which are associated to the lower risk of VTE. The methodology and the results of the meta-analysis are described in detail elsewhere (70); here only the main findings are reported.

Relevant cohort or case-control studies were searched in Medline and other electronic databases up to May 2010, with no language restriction. Data were combined using a generic inverse variance approach. Overall, the results of 55 observational studies were included. The risk of developing venous thromboembolism was significantly higher in women who use OC. The
odds ratio (OR) obtained by combining all 32 studies that reported data on the comparison between the use and non-use of OC was equal to 3.41 (95% Confidence Interval [CI]: 2.98-3.92, p <0.001). This value corresponds, approximately, to a higher risk of VTE of 3-4 times for OC users. Overall, the risk of VTE appeared slightly lower in cohort studies (OR=2.91; 95%CI: 2.33-3.62) than in case-control studies (OR=3.60; 95%CI: 3.01-4.31). Besides study design, the risk of VTE for OC users was lower in population-based studies than hospital-based studies (OR=3.31 [p<0.001] vs OR=4.19 [p<0.001]), in studies evaluating all VTE rather than idiopathic VTE only (OR=3.09 [p<0.001] vs 4.94 [p<0.001], respectively), in studies (co)sponsored by one or more pharmaceutical companies (OR=2.70 [p<0.001] vs 4.14 [p<0.001]), and in non-smokers samples (OR=2.00 [p=0.2] vs OR=5.04 [p<0.001]).

Third-generation OCs (desogestrel and gestodene) are associated with an increased risk of VTE compared to second generation (primarily levonorgestrel) (OR=1.57; 95%CI: 1.24-1.98). When the newest OCs containing drospirenone were compared to other preparations (except those containing levonorgestrel only), VTE risk did not significantly increase (OR=1.13; 95%CI: 0.94-1.35). Differences in VTE risk were also observed according to oestrogen dose: users of OC at doses ≥50 mcg show an higher risk of VTE compared to users of OC at doses <50mcg (OR=1.42; 95%CI: 1.15-1.76).

The pooled OR of the studies that have examined the risk of VTE in women with only G20210A mutation taking OC, compared to women with the same mutation but not taking OC, is equal to 1.63 (95%CI: 1.01-2.65). The overall OR of VTE for women taking OC in the population of women with FVL mutation was 1.80 (95%CI: 1.20-2.71). Women with the MTHFR mutation OC users showed a higher risk of VTE compared with non-users (OR=2.73; 95%CI 0.78-9.56), but this increase was not significant.

Based on the results of 55 observational datasets, this meta-analysis confirms that OC use is associated with a significant increase in VTE risk. The strength of this association, however, varies according to OC generation, outcome definition, presence of a genetic mutation and eventually smoking status, with relative risks varying from 3 to 5 for OC users. When compared with other available OC preparations (except those containing levonorgestrel only), the newest OCs containing drospirenone did not show a significant increase in VTE risk. As regards outcome definition, the development of methodological standards for studies on VTE is strongly warranted to reduce the variation in the estimates of singles studies, or at least to prevent misinterpretation of the strength of the association between VTE and OC use. Concerning genetic mutations, the further increase in VTE risk among the carriers of G20210A and FVL mutations prompts further evaluations of the potential implications of genetic testing.

References
(1) ACCE model, available on line at: http://www.cdc.gov/genomics/gtesting/ACCE/index.htm
(7) Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the


(10) Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism: A meta-analysis involving approximately 120,000 cases and 180,000 controls. Thromb Haemost 2009;102: 560-70


(24) Pineda LA, Hathwar VS, Grant BJ. Clinical suspicion of fatal pulmonary embolism. Chest 2001; 120: 791-5


(41) Grosse SD, Khoury MJ. What is the clinical utility of genetic testing? Genet Med 2006; 8: 448-50


(45) Jordan WM. Pulmonary embolism. Lancet 1961; 1146-7


(50) Kahn SR. The post thrombotic syndrome. Thromb Res 2011; 127 Suppl 5: S89-92


(63) Marchiori A, Mosena L, Prins MH, Prandoni P. The risk of recurrent venous thromboembolism among


4. Systematic review of the economic literature on genetic testing for the prevention of venous thromboembolism

Amelia Compagni, Alessia Melegaro, Rosanna Tarricone

4.1 INTRODUCTION

According to the ACCE framework for comprehensive Health Technology Assessment (HTA), economic considerations must be kept in mind when making decisions and formulating recommendations about genetic testing, as for other medical technologies (1). However, conducting cost-effectiveness analyses (CEA) for diagnostic and predictive testing presents several methodological challenges (2). First, diagnostic testing can affect clinical outcomes positively, but the outcome often depends on a long series of treatment decisions in which numerous additional variables come into play (3). As a consequence, it is difficult to determine the exact contribution of diagnostic testing. Second, clinical outcomes alone cannot capture the overall value of diagnostic procedures, for both patients and medical doctors, in terms of psychological consequences and knowledge (4, 5). For instance, “knowing for knowing’s sake” is one potential benefit provided by diagnostic testing (4). Finally, diagnostic testing rarely consists of one simple procedure. More often, diagnostics are associated with a plurality of tests, which are conducted concurrently and might be associated with screening programmes and counselling (6, 7). In this sense, rather than tests, it would be more appropriate to talk about diagnostic services, which are less amenable to standard HTA techniques (6, 7).

So far CEA have not been used commonly to inform policy guidelines and clinical practice with regard to diagnostic testing (8). Most tests have been introduced into healthcare systems “riding a wave of enthusiasm rather than evidence” (8). The array of genetic tests [Factor V Leiden (FVL), Prothrombin (PT) 20210A, methylenetetrahydrofolate reductase (MTHFR)] that are used to detect predisposition to venous thromboembolism (VTE) is an example. Their use is widespread at an international level and, similarly, in Italy. In such cases, to ensure that CEA of specific tests or diagnostic services are meaningful and informative, it is necessary to build credible economic models that mimic the true situation of clinical practice in a specific country as much as possible, and that are populated with solid inputs, including data on clinical effectiveness, epidemiological variables and costs.

4.2 OBJECTIVE

Before building a CEA model for VTE that was tailored to the Italian context, it was necessary to assess the existing literature on the cost-effectiveness of screening for genetic defects that predispose to VTE: FVL, and mutations in PT20210A and in MTHFR. For this purpose, we have conducted a systematic literature review and a critical appraisal of the relevant scientific publications produced so far.

4.3 METHODS

In January 2011, we searched the Medline, Embase, and NHS HTA report databases for publications on the cost-effectiveness of screening for genetic variants and mutations involved in VTE. The searches were based on a combination of several terms: (“cost-effectiveness” or “cost”) AND (“thrombophilia screening/testing” or “thrombosis” or “venous thromboembolism”) AND (“Factor V Leiden” or “Factor II” or “MTHFR”). The reference lists of all articles of interest were examined to retrieve additional relevant publications. The search was conducted separately by two researchers and their results compared. The same two researchers also read the abstracts of all the articles retrieved and excluded papers that: (i) were not based on empirical studies but only mentioned or commented on the costs or economic impacts of screening for VTE predisposition; (ii) only described cost analyses and not full economic evaluations of screening for VTE predisposition that compared alternatives.
4.4 RESULTS

The systematic review of the scientific literature identified 1229 potentially relevant papers (Figure 1). On closer inspection, only 40 were considered to be of interest, because they included economic considerations and/or studies. Among these, several were reviews or commentaries without any empirical data. Two of the articles retrieved (9, 10) did not describe full economic evaluations but only cost analyses that assessed the costs of performing the genetic tests. For this reason, they have not been included in the present analysis (Figure 4.1). Seven articles (Table 4.1) provided full economic evaluations of screening for FVL, PT20210A or MTHFR. Two of these studies (11, 12) were cost–consequence analyses because they considered only intermediate end-points (additional cost per averted VTE event), instead of life years gained or quality-adjusted life years (QALY) gained; consideration of the latter is required for CEA.

4.4.1 Combinations of genetic and biochemical tests

The seven studies that were retrieved underlined that, in clinical practice, both genetic and biochemical tests are performed at the same time in order to assess a variety of coagulation defects that predispose to VTE, some of which are inherited and others acquired.

Regarding inherited defects, two main categories of test are indicated:

(1) Genetic tests that detect FVL or mutations in prothrombin (PT) 20210A or MTHFR. All these mutations are relatively common in the general population and are correlated with an increased risk of VTE. Out of these three tests, the first two were considered in most of the studies, whereas only one article (13) included the test for MTHFR. In two studies (11, 14), the genetic test for FVL was performed subsequently to a biochemical test for activated protein C resistance (APCR), which detects a deficiency that is present in 20–60% of thrombophilic individuals and in 3–15% of the general population. Given that, in 90% of patients, APCR is associated with a mutation in FVL, this test represents the best alternative to the genetic test for FVL and may be used in a sequential screening strategy in which the genetic test is performed only to confirm the result obtained with the biochemical test (14). Marchetti et al. (15) modelled an additional sequential testing strategy, in which the biochemical test for APCR was performed first, followed by the FVL and PT20210A genetic tests for positive patients only. Another common assay, the homocysteine test, which indicates abnormal functioning of the enzyme MHTFR, was not mentioned in any of the articles under consideration.

(2) Biochemical tests that detect deficiencies in protein S, protein C, and antithrombin III. Deficiencies in these factors are caused by genetic mutations that are rather rare in the general population and for which functional assays have been developed. Four papers (11, 12, 13, 16) included these tests in a broader...
panel (sometimes called thrombophilia screening) in which they were performed together with the genetic tests.

With regard to acquired defects, the tests indicated in the articles retrieved were biochemical and used anti-phospholipid antibodies: anti-cardiolipin antibody and lupus anticoagulant. The latter test is also called the Russell viper venom assay (12, 13, 16).

On the basis of the tests performed, the seven studies retrieved could be divided into three categories. Smith et al. considered only genetic tests, and in particular that for FVL (17). The second group of articles comprised those by Clark et al. (11), Eckman et al. (14), and Marchetti et al. (15), in which a biochemical test (APC sensitivity ratio or APCR) was performed first and only patients with a positive result underwent the genetic test for FVL. Finally, Wu et al. (12), Auerbach et al. (13), and Simpson et al. (16) considered biochemical and genetic tests that were performed simultaneously. In light of these findings, it is clear that, in the attempt to build a valid cost-effectiveness model, the patterns and combinations of tests that are used might affect the result greatly and need, therefore, to be accounted for carefully.

4.4.2 Applications of screening strategies: universal and targeted

The screening strategies for defects that predispose to VTE that are presented in the articles retrieved are of two types: universal or targeted/selective. Two of the papers retrieved propose a comparison between universal and selective screening. For example, Clark et al. (11) considered the screening of all pregnant women (universal) or only those with a personal or family history of VTE (selective). Wu et al. (12) compared universal screening and selective screening for four categories of individual: women before the prescription of combined oral contraceptives, those being prescribed hormone replacement therapy, pregnant women, and patients scheduled for major orthopaedic surgery. All these situations are known to increase the risk of VTE further.

In contrast, in all the studies that modelled only selective screening strategies, the categories considered to be at risk were people with a previous episode of VTE and/or people with a family history of VTE. Smith et al. (17) considered a very limited subgroup of at risk patients: asymptomatic female relatives of FVL carriers.

The results of the studies that consider universal screening (Table 1) are variable, but they all lead to a similar conclusion: that in general this strategy is unjustified and should be highly discouraged. Clark et al. (11) reported that, in comparison to no screening for FVL during pregnancy, an additional cost of £13 281 was required to avoid one vascular event. Wu et al. (12) calculated the incremental cost-effectiveness ratios (ICERs) for all the populations indicated above. These ICERs, which are the traditional products of CEA models, were calculated as the ratio between costs (screening or no screening) and clinical complications prevented (screening or no screening). This resulted in a very broad range of costs in the universal screening strategy: from £6 800 per event averted (hormone replacement therapy) up to £200 000 per event averted (oral contraceptive).

The studies that focused instead on selective screening analysed different applications of this strategy (Table 4.1). The first group of studies (13-16) modelled how information about thrombophilia defects might affect the duration of anticoagulant therapy for people undergoing VTE. Normally, anticoagulant therapy lasts 3–6 months, whereas the simulation implied that this could be extended to 2 years, 10–20 years or lifelong therapy in such cases. Eckman et al. (14) showed that screening followed by 3 years of treatment dominated the “don’t test and standard therapy” option as well as that of “test and treat for life”. This last study considered different probabilities of recurrence of VTE, information that, given the lack of solid epidemiological data, is still uncertain and controversial in the existing literature (14). Assuming a constant rate of VTE recurrence in the years following the first episode, the comparison of “test and treat for 3 years” vs. “don’t test and standard therapy” led to an ICER of $33 900/QALY, whereas “test and treat for life” vs. “don’t test and standard therapy” gave an ICER of $16 823/QALY. In contrast, in the only economic evaluation populated partially with Italian data, Marchetti et al. (15) showed that extending anticoagulant therapy from 6 months to 2 years led to an ICER of $13 624/QALY. It should be noted that, among the seven articles retrieved, the study of Marchetti et al. was the only economic analysis to be conducted from a societal perspective; it considered indirect costs due to the patients’ loss of productivity in addition to the costs of medical treatment.

Similar results were obtained by Auerbach et
al. (13), who showed that screening and extending the duration of anticoagulant therapy from 6 months to 2 years resulted in an ICER of $11 000/QALY. Finally, Simpson et al. (16) identified the target populations in which screening following an episode of pulmonary embolism (PE) or deep vein thrombosis (DVT) would lead to ICERS below £20 000/QALY and concluded that, overall, screening appears to be a viable option. However, in the latter study, the authors identified numerous points of uncertainty in the input data (i.e. rate of VTE recurrence, sensitivity and specificity of
### Table 4.1 (Continued)

**ARTICLES RETRIEVED BY SYSTEMATIC LITERATURE REVIEW: COST-EFFECTIVENESS OF SCREENING FOR GENETIC VARIANTS AND MUTATIONS INVOLVED IN VTE**

<table>
<thead>
<tr>
<th>Article</th>
<th>Type of analysis</th>
<th>Detected mutations</th>
<th>Type of test performed</th>
<th>Type of screening (risk category)</th>
<th>Screening application</th>
<th>Results and notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu et al 2005 (12)</td>
<td>cost-consequence analysis</td>
<td>FVL and FII G20210A</td>
<td>genetic tests carried out together a panel of biochemical tests (antithrombin III, protein C and S, anticardiolipin antibody and lupus anticoagulant)</td>
<td>universal and targeted (personal and/or family history of VTE)</td>
<td>in the cases of oral contraceptives and hormone replacement therapy women found positive are not prescribed the drugs. In the case of pregnancy: antenatal and 6 weeks' postnatal heparin prophylaxis. In the case of orthopaedic surgery: extension of prophylaxis for 4 weeks</td>
<td>universal screening oral contraceptive £200 402/averted VTE; pregnancy £81 436/averted VTE; hormone replacement therapy £6 824/averted VTE; orthopaedic surgery £14 129/averted VTE. Selective screening oral contraceptive £79 085/averted VTE; pregnancy £70 254/averted VTE; hormone replacement therapy £2 446/averted VTE; orthopaedic surgery £9 136/averted VTE.</td>
</tr>
<tr>
<td>Smith et al 2008 (17)</td>
<td>cost-effectiveness analysis</td>
<td>FVL</td>
<td>genetic test</td>
<td>targeted (asymptomatic female relatives of FVL carriers)</td>
<td>women found positive for the tests are: 1. denied oral contraceptive 2. prescribed oral contraceptives but with anticoagulant treatment in high-risk situations 3. prescribed oral contraceptives but with long-term anticoagulant treatment</td>
<td>screening option dominates non screening option dominated; &quot;test, oral contraceptive and anticoagulant in high risk situations&quot; vs &quot;test no oral contraceptive&quot; = $147/QALY; &quot;test, oral contraceptive and long-term anticoagulant&quot; vs &quot;test, oral contraceptive and anticoagulant in high risk situations&quot; = $639 500/QALY</td>
</tr>
<tr>
<td>Simpson et al. 2009 (16)</td>
<td>cost-effectiveness analysis</td>
<td>FVL and FII G20210A</td>
<td>genetic tests carried out together with a panel of biochemical tests (antithrombin III, protein C and S, anticardiolipin antibody and lupus anticoagulant)</td>
<td>targeted (previous episode of VTE)</td>
<td>extension of anticoagulant therapy (warfarin) from 3 months to 10.20 years or lifelong treatment after VTE</td>
<td>cost/QALY=&lt; £20 000/QALY for most target populations (sex, age) and after both PE or DVT</td>
</tr>
</tbody>
</table>
thrombophilia tests) and remained cautious in their recommendations for clinical practice.

The second group of studies that focused on selective screening (11, 12, 17) dealt with cases in which information about thrombophilic defects affected clinical decisions and prescribing behaviour. The decisions affected were not specifically in relation to the treatment of VTE, but rather to defined clinical interventions in high risk situations such as pregnancy, as well as to the administration of oral contraceptives, hormone replacement therapy, and anticoagulant prophylaxis before major orthopaedic surgery. For instance, Clark et al. (11) focused on pregnant women who were prescribed anticoagulant therapy before and after delivery upon testing positive. In this study, the incremental cost per prevented VTE was calculated to be equivalent to £7 535. In contrast, Wu et al. (12) obtained a very broad range of results with respect to the application of their screening strategy: from £2 446/averted VTE for hormone replacement therapy to £79 085/averted VTE in the case of oral contraceptives (Table 4.1). The study assumed that women who tested positive would not be prescribed the treatment under consideration (oral contraceptive, hormone replacement therapy) or that, as a result of the information from screening, thromboprophylaxis would be extended (in the case of major elective orthopaedic surgery). The article concluded that, although selective screening reduced the cost of avoiding one vascular event in certain situations, such as in women given oral contraceptives and during pregnancy, the overall cost of these strategies (£70 000–75 000 per event averted) meant that they remained highly unfeasible. Finally, Smith et al. (17) assumed that women who tested positive for the genetic mutations either would not be prescribed an oral contraceptive, as in the previous article, or would undergo treatment with anticoagulants either in high risk situations (e.g. during flight, surgery, immobilization) or for a long period of time (i.e. 15 years). The results showed that “no screening and usual care” was dominated by all screening strategies. In addition, the strategy “screening, contraceptive and anticoagulant in high risk situations” vs. “screening, no contraceptive and no anticoagulant” led to an ICER of $147/QALY. Finally, extending anticoagulant therapy from during high risk events to lifelong therapy led to an ICER of $639 500/QALY. Overall, the article concluded that in a very limited population of women at risk, testing and prevention of VTE by targeted anticoagulant therapy was actually cost-effective and would be preferable to denying the contraceptive altogether. It should be noted that this was the only article that included the consequences of unwanted pregnancies in women who had been denied the contraceptive pill.

Although they were not considered in the critical appraisal, the two cost analyses (9, 10) that were retrieved during the systematic literature review provided some additional information. Palareti et al. (9) investigated screening for congenital thrombophilic alterations among women who requested prescription of an oral contraceptive, and estimated that the total cost to detect one altered case is $7 795 for protein S, $2 696 for antithrombin III, $1 374 for protein C, and $433 for APCR. However, Creinin et al. (10) showed that over $300 million would be spent and 92 000 FVL carriers identified to prevent one death due to VTE among women using oral contraceptives.

In Table 2, the studies have been categorized into Groups 1 and 2, on the basis of the applications of genetic tests they describe. In Group 1 tests are used to define the anticoagulant therapy in case of VTE; in Group 2 tests are utilized to prevent VTE in high risk situations such as pregnancy, oral contraception, hormone replacement therapy and major surgery.

4.4.3 Critical appraisal of the economic literature

To carry out a critical appraisal of the literature we considered the checklist proposed by Drummond and colleagues (18) (Table 4.2). In addition, given that, as explained in the introduction, these tests are already widely used in clinical practice, we focused on two aspects that, in our view, are particularly important in determining the credibility and robustness of these cost-effectiveness models: (i) the validity of the reconstructed decision tree, and (ii) the sources of the input data, such as test accuracy, costs, and epidemiological data. Improving the credibility and robustness of these models should bring them closer to the real clinical practice that they are designed to modify and inform.

Concerning the decision tree, the article by Wu et al. (12) was the only one in which the clinical decision-making process was obtained through two Delphi rounds and a survey on healthcare professionals, in this case conducted...
among a group of approximately 100 consultants in obstetrics and orthopaedics in Scotland. The rest of the articles based their theoretical modelling mainly on the available literature and clinical guidelines.

In addition to the means by which the decision tree was obtained, another aspect that might have influenced the quality of the decision modelling is the way in which the categories of the population who are at risk of VTE were considered. In most of the papers retrieved, these categories comprised individuals with a personal or family history of idiopathic VTE, which are both potential indicators of inherited thrombophilia. In the articles in which both groups (personal and family history) were considered, they were modelled together, although it is unclear whether the probability of a first episode of VTE (in those with a family history of VTE) and the probability of recurrence of VTE (in those with a personal history) are exactly the same or, at least, are perceived to be the same by medical doctors. In addition, Eckman et al. (14) and Simpson et al. (16) indicated clearly that the rate of VTE recurrence is in itself controversial, and for this reason they modelled different scenarios of this rate, and reached quite different results. Finally, although a familial history of VTE points to a case of inherited VTE, a previous episode does not, and this might affect the prescribing behaviour of clinicians with regards to tests. In other words, the existing CEA models group together populations...
that are at risk of VTE and tend to neglect the fact that the clinical decision-making process might be influenced by whether the VTE episode to be prevented through testing is potentially caused by inherited thrombophilia or is acquired.

With regard to clinical effectiveness, the clinical and epidemiological data used as inputs for the CEA models in the studies retrieved were taken in general from the literature, although with varying degrees of thoroughness. For instance, Wu et al. (12) and Simpson et al. (16) conducted very comprehensive analyses of the major studies that presented the epidemiology of VTE and of the meta-analyses used to calculate the risks associated with the genetic defects of interest. In contrast, Clark et al. (11) evaluated the effectiveness of testing in parallel with a prospective study that involved almost 1,000 pregnant women. As a consequence, this was the only study with primary data.

Furthermore, clinical effectiveness was evaluated in different ways in these papers. In two articles, the end-points used were measured as “averted cases of VTE”. Prevented events are intermediate end-points. A complete economic evaluation should normally express clinical benefits in terms of life years gained or QALY gained. In these two articles, it was assumed that averting a VTE event would have an impact on final outcomes such as life expectancy. However, this causal relationship has not been proven or calculated. Using intermediate outcomes makes it difficult, if not impossible, to compare the current technology with other technologies. For CEA to be a valid instrument in the decision-making process for the allocation of scarce resources, it needs to provide policy-makers with consistent outcomes. It is impossible to rank and prioritise technological innovations whose benefits have been measured using different units.

In all the studies, another uncertain input in the CEA models was the accuracy of the tests employed in screening. Not only were the tests assembled in one test panel and, thus, given an average accuracy, but also most sensitivity and specificity data were theoretical and not determined by analysing the laboratory practice in the specific context under study.

Finally, with regard to the cost data, six out of the seven studies retrieved considered only direct costs, whereas Marchetti et al. (15) calculated both direct costs and productivity losses. With regard to the mix of sources used by these authors to retrieve cost data (Table 4.3), the variability in parallel with a prospective study that involved almost 1,000 pregnant women. As a consequence, this was the only study with primary data.

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<table>
<thead>
<tr>
<th>Article</th>
<th>Type of costs</th>
<th>Source of costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marchetti et al 2001</td>
<td>direct and indirect</td>
<td>Test: local laboratory practice; VTE treatment: Italian list prices and charges and other economic analyses conducted in Italy; discharge costs from Spain; Indirect costs: assumptions about days off work and San Valentino Venous Disease project (Italy)</td>
</tr>
<tr>
<td>Clark et al 2002</td>
<td>direct</td>
<td>NHS costs database; British National Formulary</td>
</tr>
<tr>
<td>Eckman et al 2002</td>
<td>direct</td>
<td>Average Medicare DRG reimbursement; Outpatient cost data were o from the Tufts Associated Health Plans</td>
</tr>
<tr>
<td>Auerbach et al 2004</td>
<td>direct</td>
<td>Costs of tests: Clinical Diagnostic Laboratory Fee Schedule; costs of bleeding and thrombotic events: average inpatient DRG reimbursement, adjusted for inflation and average length of stay</td>
</tr>
<tr>
<td>Wu et al 2005</td>
<td>direct</td>
<td>National Health Service in Scotland; Hospitals NHS Trust; Clinical Services Division, Laboratory Directorate, North Glasgow University</td>
</tr>
<tr>
<td>Smith et al 2008</td>
<td>direct</td>
<td>Literature</td>
</tr>
<tr>
<td>Simpson et al 2009</td>
<td>direct</td>
<td>Literature and UK NHS reference costs database</td>
</tr>
</tbody>
</table>
4.5 DISCUSSION

Some elements of interest have emerged from the analysis of the literature described above. Good CEA studies of VTE have been published, and useful models have been developed on this topic. Although the results of these studies are not always comparable and in agreement, genetic screening for VTE appears to be cost-effective only for targeted populations who are already at risk (with a personal or familial history of VTE), especially when the risk is augmented further by specific conditions such as pregnancy, the use of oral contraceptives, or surgery. It is unclear from the literature whether other behaviours (e.g., diet, smoking) are also important modulators of the risk of VTE and whether they should be considered by clinicians when deciding on the suitability of testing. Even for cases of certain relevance to public health, such as those of women prescribed an oral contraceptive, a general consensus on whether to proceed with testing has yet to be reached in most countries.

The existing studies have attempted to encompass all the benefits and consequences of testing for predisposition to VTE. However, it is not easy to draw a comprehensive picture. For instance, with respect to screening in association with the use of oral contraception, one of the possible consequences is that women who test positive will be denied the contraceptive. In making this decision on the basis of the test results, gynaecologists need to balance the risks carefully, because, in principle, an unwanted pregnancy leads to an even higher risk of VTE than the use of an oral contraceptive. In addition, this decision might affect the patient-clinician relationship negatively, an aspect that is difficult to evaluate.

Apart from one case, most of the studies reviewed were based on theoretical modelling. Given that tests for predisposition to VTE are employed commonly in most countries, we suggest that, to generate a consolidated clinical practice, CEA models should be tailored better to the real decision-making processes followed by physicians. In this way, cost-effectiveness analyses could help clinicians to envisage the consequences of their decisions, with respect to both the epidemiology of the disease and the consumption of resources, and support them in targeting testing more effectively to even smaller populations, whose risk of VTE physicians can assess more confidently.
References


(2) Drummond M, Griffin A, Tarricone R. Economic evaluation for devices and drugs - same or different? Value in Health 2009; 12: 402-6


(5) Lee DW, Neumann PJ, Rizzo JA. Understanding the medical and nonmedical value of diagnostic testing. Value in Health 2010; 13: 310-4


(15) Marchetti M, Quaglini S, Barosi G. Cost-effectiveness of screening and extended anticoagulation for carriers of both factor V Leiden and prothrombin G20210A. QJM 2001; 94: 565-72


5.1 Ethical considerations

Maria Luisa Di Pietro, Adele Anna Teleman

5.1.1 INTRODUCTION

Venous thromboembolism (VTE) is a common source of morbidity and mortality, which clinically manifests itself as either deep venous thrombosis (DVT) or pulmonary embolism (PE). According to a European study, the incidence per 1 000 persons per year was estimated to be 1.48 for DVT and 0.95 for PE (1). There is no available data at the moment on the incidence of VTE in Italy due to the fact that there are no specific studies on the subject, and that it is difficult to compare the existing studies since they do not consider homogeneous populations (2).

Many intrinsic factors (age, obesity, previous history of VTE), disease-related risk factors (i.e. lupus anticoagulant or anticardiolipin antibodies), physiological (pregnancy) and iatrogenic factors (oral contraception, hormone replacement therapy) can increase the baseline propensity to develop VTE (3). In adults with a history of idiopathic VTE, the events occur in the absence of a known precipitating factor, but in the presence of an inherited risk factor. In fact, in addition to the factor V Leiden (FVL) and Prothrombin (PT) 20210A gene mutations, heritable causes of VTE include deficiencies in natural anticoagulants such as antithrombin, Protein C, and Protein S (4). Among risk factors for VTE, there is the elevation of homocysteine level, that is - in part - under the control of methylenetetrahydrofolate reductase, coded by the MTHFR gene. Its functional variant C677T has been considered a relevant factor for VTE and is included in a panel of genetic testing (5).

The FVL, PT20210A and MTHFR mutations testing offers today the possibility of detecting the increased risk of thrombosis with the aim of a preventive intervention. These tests currently represent in Italy a fourth of all the genetic tests conducted on adults (6).

They represent an important public health cost also due to the increasing trend in their frequency. Even after having proven their analytical validity, clinical validity and clinical utility, it is still necessary to question if, within a context of limited economical resources, their use is justified in public health.

An answer to this question can be found through a process of Health Technology Assessment (HTA), that takes in consideration not only the technical, clinical and economical aspects (7), but also the social and ethical ones (8-10). On the other hand, it is by now a given fact that the ethical evaluation is an integral part of a HTA process. HTA has been defined as “a multidisciplinary field of policy analysis. It studies the medical, social, ethical and economic implications of development, diffusion and use of health technology” (11).

Considering that the objective of HTA is to bridge between the scientific world (research) and the political one (the decisional process), an ethical contemplation will fill in the gap left by technology. In fact, even if technology is capable of responding to questions as regards to the safeness, efficacy, efficiency, and economical impact, it is not capable of responding to ethical questions (what dangers does this technology hide? Can it induce false hopes? Does it take in consideration the fragility and the vulnerability of humans?)

Even the ACCE protocol - which has been created for the evaluation of genetic tests - determines that the elements to take in consideration are not only the analytical validity (A), the clinical validity (C) and the clinical utility (G), but also the ethical, legal and social aspects (ELSI), which are an integral part of the decision-making process (12, 13).

As opposed to other domains in which there are methodologies that are rather uniform, ethical evaluations depend on the criteria and methodologies of the different currents of thought. This analysis is based on the cognitivist ethics, which acknowledges that it is possible to reach some truths regarding man and his actions, which are, generically, recognizable by everyone. As for the methodology, the study is developed in three moments: the epistemological moment, the anthropological moment and the ethical moment.

5.1.2 THE EPSTEMOLOGICAL MOMENT

As mentioned before, the genetic polymorphisms that most commonly predispose to VTE are prothrombin (PT) 20210A, factor V Leiden...
G1691A (FVL) and Methylene tetrahydrofolate reductase C677T (MTHFR). Genetic tests that evaluate the personal susceptibility to VTE make it possible to start a prophylactic treatment, with an anticoagulant therapy, in adults with a personal history of VTE or in adult family members of mutation-positive individuals, in order to improve clinical outcomes.

Based on the available data, we can deduce that, while the analytic validity for both FVL and PT20210A is high, the clinical validity of the tests is variable. As regards to the clinical utility, in adults with a history of VTE, there is no direct evidence that these tests lead to improved clinical outcomes (14, 15). In fact, evidence proves that long-term secondary prophylaxis, after an initial idiopathic VTE event, yields comparable benefits to those with or without a FVL or PT20210A mutation. As regards to asymptomatic family members of patients with VTE, there is no evidence that knowledge of a FVL/PT20210A mutation, and consequent anticoagulation treatment, is actually useful for avoiding initial episodes of VTE (16).

Taking in consideration also MTHFR C677T, it has been demonstrated, by a meta-analytical study, that women, who have a FVL, PT20210A or MTHFR C677T mutation and that take oral contraceptives, have a significantly higher risk of developing VTE (17).

5.1.3 THE ANTHROPOLOGICAL MOMENT

In order to develop an ethical evaluation within the HTA, we must first clear the anthropological reference (18). In fact, if we were to use a merely procedural approach, we would not be able to rationally justify the moral values, principles and norms, and this would lead us to arbitrary results (19). In every case, we must always find a solution that reflects the ultimate reasons (moral values, principles) that conduct towards a choice.

As stated before, this ethical analysis is based on a cognitivist ethics, which rotates around an unconditional respect of the human being. As a consequence, the defense of physical life, the promotion of health and of the quality of life, the respect of free and responsible choices, the search for the common good, are all fundamental values which create a hierarchic structure. Within the biomedical field, this approach translates into the evaluation of the consequences that the use/introduction of a health technology can have on the integral well-being of the human.

5.1.3.1 Assessing benefits and harms

The benefit which is sought through the use of genetic tests for the FVL, PT20210A, MTHFR C677T mutations, is the possibility, in case of an increased risk, to start an anticoagulant therapy, in order to prevent episodes of VTE. Even though the analytical validity is high for these tests, we cannot say the same for the clinical validity and the clinical utility. Scientific evidence proves there is an advantage only for subjects who have homozygote mutations for FVL or PT20210A, a condition which is very rare in the population and usually interests people of a young age (<45 years) (20).

Genetic testing can, on the other hand, lead to medical and psycho-social harms. For example, there is an iatrogenic risk if primary prophylaxis is administered to asymptomatic family members that have one or more mutations. In fact, the absolute risk of an initial VTE event is low, while the risk of anticoagulant-induced hemorrhage is relatively high (16).

Among the psycho-social implications of predictive genetic tests are: the effect that they can have on personal identity, the ambiguities in the concept of genetic disease and the fact that genetic information is a kind of information that also regards others (21). In fact, predictive genetic tests bring to the knowledge of genetic makeup and inheritance which can affect personal identity, since it influences physical attributes and traits, and propensities towards disease. Genomic knowledge can, therefore, in itself, be the cause of a raise in anxiety and distress (22).

Genetic testing - which may predict diseases long before the manifestation of any symptom or susceptibility - also challenges the notions of disease (23). How should we consider this person - ill, healthy, an “asymptomatic ill” or an “unpatient” - since he/she will, could or should develop the symptomatic disease in the future? The detection of a predisposition to a genetic disease can promote the view that a person is “in actual fact” already sick, determining, as a result, an adverse effect on that person’s zest for life and general behavior. Yet, genes are only one of the many factors contributing to health, which, in a more holistic approach, is the product of the interaction between somatic, psychological and
spiritual elements. Health is, in reality, the result of the interaction of the body-mind system with the environment.

Finally, genomic knowledge is different from other forms of knowledge since it can be, by definition, information also about others. It can be relevant to the person’s biological relations, since they may be carriers of, or at risk for, the same disease (24). This shows how confidentiality, an important value belonging to the personal sphere, can be difficult to maintain within a family.

5.1.3.2 The quality of life

After having proven the real clinical utility of these tests, which determine the genetic susceptibility of developing VTE, we could prevent the development and representation of medical situations that are highly invalidating, and that way, obtain an increase in quality of life. However, in the overall evaluation of the quality of life, we must consider - as already mentioned - also the possible harm that could derive from an excessive emphasis on genetic differences, and from the impact that genetic knowledge can have on personal identity (25).

Detecting certain genetic traits can, in fact, form the basis for discriminating persons and groups within the population, with the possibility of outright discrimination as a result. It would be very easy to fall into the temptation of asserting that there are differences among groups, and that those differences are genetic in nature. When such differences are used as reasons for treating people differently or as explanations for enduring inequalities, the potential for injustice is great. So the most important risk is the “geneticization”, that identifies persons with their genes and overemphasizes the role of genes in disease etiology, in medical practice and in social attitudes towards disease (26).

5.1.3.3 The respect of autonomy

The personal dimension of genetic testing implies the respect for an individual’s autonomy (27). This comprises the right to make autonomous decisions about one’s health care and to voluntarily pursue genetic testing that can have possible consequences on that person’s life.

Respect for an individual’s autonomy requires that the person himself authorize genetic testing intentionally, freely and based on understanding. Therefore, it is important that there not be pressure from the family, the professionals involved or other persons, since control by third parties would invalidate the consent given. The choice of whether or not to pursue genetic testing belongs to the individual.

Respect for an individual’s autonomy is ensured by obtaining adequate informed consent from that person, and this means more than simply a signature on a piece of paper. In fact, it is essential to offer pre-test counselling, both to evaluate the individual’s capacity for autonomous decision-making and to provide a realistic view of the test’s implications (the risks and benefits, the efficacy, the alternatives, the seriousness and potential treatment of the disorders, as well as the social and ethical implications involved) (28).

It is necessary to explain that genetic knowledge has an individual, predictive and probabilistic nature, and, furthermore, that the results of genetic testing have implications not only for the patients, but also for their biological kin (29). Genetic counselling should be carried out before submitting the individual to the test, as well as after the test, when the results are disclosed.

Finally, respect for an individual’s autonomy also entails that all information acquired through genetic testing should be considered in a confidential setting, and should not be disclosed without the individual’s consent. There could be, however, some valid reasons to breach confidentiality and to inform relatives or third parties (for example, when there is high probability of irreversible harm that disclosure will prevent, and there is no other reasonable way to avoid the harm).

On the other hand, genes are - as above mentioned - in the ‘public domain’. They are shared with others (parents, children, siblings, etc.) and it is possible to have a genetic disease or susceptibility in common with others, without any of the parties knowing it. Thus, a person’s autonomy is not appreciated in its full sense if it does not encompass that person’s responsibility towards others who are somehow involved in his/her decisions.

This concept, which is valid for all bioethical issues, is particularly crucial in the area of genetics. In fact, an individual’s awareness of his/her own genetic disease or susceptibility may entail the knowledge that relatives may also have the disease or a great likelihood of developing it. Similarly, a relative’s wish to know whether they
carry a genetic disease or susceptibility to disease may lead that individual to obtain knowledge of his/her own genetic disease or susceptibility.

5.1.3.4 Decision making and scarce resources: a problem of justice

The economical evaluation within the HTA has to take in consideration on one hand the costs of the technology in use, and on the other hand, the direct health costs (cost of collecting specimens, laboratory testing, counselling, follow-up testing, treatment and prevention), the direct non-health costs (sufferings, pain, loss of self-sufficiency) and all other indirect costs (for example, the loss of working hours) (30).

Genetic testing can determine financial benefits, when it prevents expensive medical conditions or when it avoids disease surveillance in mutation negative relatives. As regards to the genetic tests for the FVL, PT20210A and MTHFR C677T mutations, it will be necessary to determine if their analytical validity, clinical validity and clinical utility are sufficient to justify their economical cost. If it is justified, the above mentioned genetic tests must be guaranteed not only to patients with risk factors, but also to their asymptomatic relatives.

5.1.4 THE ETHICAL MOMENT

Given the incoherent results currently available, it is necessary to conduct further studies on the clinical validity and utility of the genetic tests for the FVL, PT20210A and MTHFR C677T mutations. This said, it is even more appropriate that the above mentioned tests be accompanied with adequate counselling.

For this reason, anyone who is offering (or referring for) genetic testing must provide (or refer for) appropriate genetic counselling before and after testing (31).

Genetic counselling is the only context in which to help people cope correctly with such health issues (32). Therefore, it is essential that primary care practitioners and allied health professionals have a minimal basic understanding of medical genetics and counselling.

One of the principles underlying the methodology of counselling is non-directiveness (33). It implies that professionals should not present any decision as more correct or advantageous for a person or society. However, is it acceptable, in the name of non-directiveness, to place all the options on the same level, leaving the choice solely to the individual? Is this really what individuals expect? Should the counsellor engage in nondirective counselling and only present all the alternatives, without advising for or against any choice? Or does the counsellor have the responsibility of presenting his/her moral view? The response to these questions derives from the consideration of the normativity of medicine. Medicine regards itself ultimately as a helping and healing profession. In such a concept, value-neutrality is not an appropriate position to guide medical activities. In fact, according to this view, physicians adhere to professional norms that go beyond the neutrality of values (34). Therefore, the norm of non-directiveness in clinical human genetics is inadequate also from a medical point of view. The normative attitude of clinical geneticists should shift from neutrality to prescriptivity.

Thus, if there are options that do not ensure the respect for human life, health and dignity, the counsellor has the duty to make them known, since this constitutes a part of the truth (not only the scientific truth) that he/she is called to bear witness to, as a professional and as a person (35).

There are two phases in genetic counselling: pre- and post- test counselling. An adequate understanding of the implications of genetic testing for FVL, PT20210A or MTHFR C677T mutations is a prerequisite for the tests. It is also necessary to evaluate the individual’s capacity for autonomous decision-making and, in cases where there are significant doubts concerning their capacity, to eventually postpone the test.

Pre-test counselling for FVL, PT20210A or MTHFR C677T mutations should include: (i) exploration of all pros and cons of testing; (ii) the elucidation of an individual's motives for the testing; (iii) identification of areas in which the individual's expectations may be unrealistic; (iv) understanding that the predictive value a pathological gene mutation has not been established completely; (v) avoiding the so called 'therapeutic illusion' (namely, the belief that predictive genetic testing guarantees early detection and/or prevention of disease); and (vi) information about psychological, familial, social, ethical aspects and economic consequences.

Since information on genetic testing for FVL, PT20210A or MTHFR C677T mutations associated with increased risk of VTE may be very complicated, it should be correct, complete and
communicated in a comprehensible manner. In signing the consent form, the patient is asked to state that they fully understand the terms and have had adequate opportunities to ask questions.

Finally, an important issue is whether asymptomatic minors, who are at high risk of future VTE because of their family history, or minors with thrombosis are eligible for FVL, PT20210A or MTHFR C677T mutation tests (36, 37). Genetic testing of minors is generally not advisable due to a variety of medical, ethical and psychological concerns, unless the test has a diagnostic purpose or there is a possibility to commence treatment immediately (38). For example, in familial adenomatous polyposis coli, early treatment can reduce morbidity and mortality in carriers of the mutation and eliminate the need for periodic surveillance of the colon in children who are found to not carry the familial mutation (39). For VTE, the absence of sure evidence regarding the clinical utility could discourage the recourse to genetic testing in asymptomatic minors and require the use of prudence in the case of minors with thrombosis. The question is not whether a minor is competent to consent, but whether the potential burdens of testing for FVL, PT20210A or MTHFR C677T mutations outweigh the benefits.

In post-test counselling for FVL, PT20210A or MTHFR C677T mutations, the counsellor should communicate the test results and help patients understand them. Having this aim in mind, it could be useful to evaluate through a questionnaire if the patient has obtained an adequate level of comprehension and of representation of the genetic risk (40).

It is also necessary to remember that the tested patients have the right to decide not to be told what the test results are (41). The great majority of people think that genetic testing would be a good idea and, when asked hypothetically, that they would want to be tested themselves. However, when genetic testing is really offered, the uptake is considerably lower. Even among families at high risk for genetic disorder, many individuals choose not to know. However, the right to know is of value especially for patients themselves, so that they may know what their own genetic constitution is and hence make responsible choices concerning their future lives.

There are also issues stemming from the responsibilities (parental, social) that are engendered by the right to know the genetic makeup of another person. In contrast, the right not to know is sustained by various arguments: (i) knowledge can cause distress, even if it has been observed that the benefits of knowledge could outweigh the disadvantages, and that uncertainty can also cause anguish; and (ii) since the human condition is by nature one of limited knowledge, it does not make sense to say that we ought to know or that there is a duty to know.

It would thus seem more ‘human’ to assert a right to hope versus a right to certainty. Nevertheless, an apparent contradiction remains: how could a person decide not to know without knowing what there is to know? The moral problem, in conclusion, lies not so much at the level of wanting or having a duty to know or not to know, but rather concerns how to make meaningful use of the available genetic information (42). This points to the importance of adequate counselling, at the end of which the subject may even decide not to take the test. In fact, the information obtained by the test could be so inconclusive and probabilistic that the person involved could be unable to take any subsequent measures.

If a patient has decided to know, he/she becomes the object of information. Therefore, confidentiality and privacy are important in genetic testing, and not only because of the possibility of discrimination, but also because they are crucial to preserving a person’s autonomy. In some cases, there could be others who may be interested in information for other reasons; in these cases, there is a conflict between autonomy and responsibility towards others.

For example, blood relatives or other family members have every right to be informed (43). We think there could be some good reasons to breach confidentiality only to inform relatives. In fact, high-risk family members’ access to genetic testing is usually dependent on relatives who have already had VTE and underwent mutation testing. Thus, testing individuals plays an important role in generating genetic information for their biological kin, to the point that doctors should consider the patient and his blood relatives as a unit of care.

Counsellors usually invite tested patients to disclose this information to biological kin who could benefit from the information. The ethical dilemma often faced by patients is not whether they should disclose genetic information to their relatives, but how and when they should put this in practice.

What if the patient refuses to disclose? The choice whether or not to inform relatives at high
genetic risk against a patient’s wish (or without his/her consent) is ethically difficult. In fact, the duty to preserve confidentiality is in conflict with the responsibility to warn third parties of possible harm. It is necessary to also consider if there is no preventive intervention that can effectively reduce mortality and morbidity among carriers.

In concrete cases it is necessary to weigh the risks against the harms. The US President’s Commission’s recommendation can be helpful in this. It states that disclosure is possible if there are four conditions: “a. reasonable efforts to elicit voluntary consent to disclosure have failed; b. there is a high probability both that harm will occur if the information is withheld and that the disclosed information will actually be used to avert harm; c. the harm that identifiable individuals would suffer if the information is not disclosed would be serious; d. appropriate precautions are taken to ensure that only the genetic information needed for diagnosis and/or treatment of the disease in question is disclosed” (44).

The second aim in post-test counselling is medical management. In this phase, the information offered is essential, because of the individual differences in the perception of risk and of the consequences of the choices which are made. The medical case management of the genetic tests for VTE can be complex because of the not homogeneous data regarding their clinical utility. For this reason, further clinical studies would be necessary.

### 5.1.5 CONCLUSIVE CONSIDERATIONS

In order for the discovery of such mutations not to engender stigmatization of, or discrimination against, the individuals carrying them, an ethical analysis founded on the value and centrality of the human being is essential, united with efforts directed at educating people.

It is important not only to help people understand the differences between mutation and disease, risk assessment, susceptibility penetrance, polygenicity, the interaction between genes and environment, the possibility of false negatives and false positives in genetic testing, but also to help people make choices responsibly. For this reason, education should focus on scientific facts, but it should also encompass psychological, social and ethical aspects. The education of patients lies in the hands of family physicians, who should act as intermediaries between the patients and the genetic services.
5.2 Genetic testing for inherited thrombophilia: the patients’ perspective

Vera Uliana, Alessandro Cocchella, The Project Unit Investigators, Emilio Di Maria

5.2.1 INTRODUCTION

Though *MTHFR* genetic testing is no longer recommended in thrombophilia screening according to international literature, genotyping of the *MTHFR* C677T polymorphism is still frequently ordered by prescribers as an element of the thrombophilia screening panel. The latest survey on Italian genetic laboratories showed that the sum of the assays carried out for FVL, PT20210A and *MTHFR* C677T represents the second indication for genetic testing (43001 tests per year), corresponding to the 25% of the molecular genetics tests offered in post-natal age (45). Thus, we refer to the combination of FVL, PT20210A and *MTHFR* C677T as “genetic tests” for thrombophilia from hereafter, if not differently stated.

A substantial body of literature addressed the appropriate use of genetic testing for thrombophilia, and several recommendations were produced (46-49). However, the so-called ethical legal and social issues (ELSI) associated with genetic test for thrombophilia were not deeply explored. The ACCE model (Analytic validity, Clinical validity, Clinical utility, ELSI), as originally proposed, acknowledged a primary role for the issues related to the impact of genetic tests on patients and families, and to the social context in which tests are delivered (50, 51). In the light of the actual application of the model, a newer version of the paradigm confirmed the central role of the client-related issues, and included them within the clinical utility element (52).

Within the framework of a multicentre health technology assessment project which used the ACCE paradigm to evaluate genetic testing for susceptibility to VTE, we focused on the acceptability of the test, in order to estimate the impact of test on patients and their families and to evaluate to what extent the procedure meet their expectations.

5.2.2 MATERIALS AND METHODS

5.2.2.1 Participants

Between March 2010 and June 2010, 182 consecutive individuals undergoing blood sampling for thrombophilia screening at the Galliera Hospital in Genova, Italy, were informed of the study and asked to participate. One hundred-fifty-two individuals gave written consent and were enrolled into the study. During the first contact, the following data were collected: age, indications for testing, prescribing doctor, whether the patient underwent pre-test genetic counselling or not, and preferred modalities for re-contact. One month after delivery of test results, all enrolled individuals were re-contacted via phone, e-mail or ordinary mail, according to their preference, and the study questionnaire was sent in the proper form (electronic form or hard-copy). A reminder was sent twice to those who did not respond after one month. Ninety-seven questionnaires were collected (response rate: 97/152, 64%).

5.2.2.2 Study instrument

After an inspection of all questions listed in the ACCE check-list (51), we selected question 27 (“What is the impact of a positive (or negative) test on patient care?”) as the most pertinent. A questionnaire was developed to explore the possible answers to this question, and to estimate some of the consequences of thrombophilia genetic testing. The questionnaire was designed in Italian. After an extensive inspection of relevant literature, the questionnaire was drafted based on previous studies, by adding specific items focused on the aim of the research. Before administration to the patients’ cohort, it was tested on a small group of health professionals and eventually amended. No formal validation procedure was applied. The systematic literature search and the detailed description of the
study questionnaire will be reported elsewhere [manuscript in preparation]. In brief, the threepage questionnaire contained an initial section about demographics (age, gender, ancestry, education) and a specific section. The latter consisted of 14 items, addressing the following questions: i) knowledge about the indications for thrombophilia genetic test and about test results; ii) reactions to test results and perceived health risk; iii) offer of genetic counselling and patient satisfaction about it; iv) consequences of genetic testing (changes of clinical pathway, cascade testing in relatives). Questions were primarily closed-ended. The final version and its translation in English are available on request.

Statistical analysis was performed using Stata 9 (StataCorp LP, College Station, TX, USA). The threshold for significance was set at 0.05.

5.2.2.3 Genetic analysis

FVL, PT20210A and MTHFR C677T were analysed by mean of an assay based on polymerase chain reaction and reverse hybridisation (FV-PTH-MTHFR StripAssay, ViennaLab Diagnostics, Vienna, Austria), according to the manufacturer’s instructions. Stringent quality assurance procedures were applied throughout the entire workflow.

Test results were manually extracted from medical records. If not defined otherwise, individuals heterozygous or homozygous for: i) FVL, or ii) PT20210A, or iii) MTHFR C677T, were defined as carriers of inherited thrombophilia. Individuals who did not present such a genetic test result were defined as non-carriers, irrespectively of the number of tests (from 1 to 3).

5.2.3 RESULTS

The sociodemographic characteristics of the participants are shown in Table 1. Both the enrolled cohort and the subset of responders to the questionnaire were considered. Women were the majority in both series, consistently with the reported indications for testing. After personal history of thromboembolism, prescription of oral contraceptives is the most frequent single indication. Moreover, less frequent indications such as pregnancy or recurrent abortions pertain only to women. The sum of family history for VTE (28/152) and positive genetic test in a relative (13/152) results in the most frequent indication (26.9%). Comparing the cohort of participants to the series of responders, chi-squared statistics did not reveal significant difference. A minority of participants underwent a genetic counselling session before testing (Table 5.1).

Table 5.2 reports the genotype frequencies for FVL, PT20210A and MTHFR C677T in the patients’ cohort and in responders to the questionnaire. Carriers were 29.6% and 29.9% (45/152 and 29/97), respectively. Two-sample test of proportion did not reveal any difference in genotype frequencies between the whole cohort and the subset of responders. Consequently, we considered the subset of responders as a random sample of the whole cohort.

On average, 95% of patients recalled the test results, with a slightly higher percentage in carriers (96.6%). Based on test results, 51.7% and 6.9% of carriers stated to have a higher than average probability, and much higher probability, respectively, to suffer from a thromboembolic event, as compared to the majority of non-carriers who attributed to themselves the same probability of general population, or lower (44.1% and 32.3, respectively).

The distribution of answers to the question “what was your reaction to the test results?” clearly differed between carriers and non-carriers. Most carriers (20/29, 69%) declared to have felt slightly or severely insecure after the test disclosure, as compared to the 88.2% of non-carriers who declared no consequence. The item exploring the middle-term impact of test results ("how often have you thought about your risk of thrombosis after test disclosure") showed a smaller difference in answers distribution, as 48.3% of carriers reported a possible mild worry ("sometimes") versus 30.9% of non-carriers.

About one-fourth of carriers (8/29, 27.6%) were suggested to undergo a genetic counselling visit after test result (4.4% of non-carriers). Only six patients underwent the visit before the interview, therefore the results from the question exploring their satisfaction after genetic counselling was not further analysed.

One item explored changes in clinical path after genetic testing. Most carriers reported to have been prescribed a clinical follow-up (34.5%), or a pharmacological treatment (15.8%), or further investigations (6.8%). Notably, 37.9% of carriers (11/29) reported that no clinical intervention had been planned. This answer was given by 63.2% on non-carriers, but 16.2% of them (11/68) reported to have been prescribed further clinical
intervention; particularly, a drug prescription was reported by 7.4% of non-carriers. Most responders shared the test result with relatives (75.9% of carriers and 79.4% of non-carriers). At the time of survey, 20.7% of carriers (6/29) and, remarkably, 10.3% of non-carriers (7/68), reported that thrombophilia genetic testing had been performed in relatives.

### 5.2.4 DISCUSSION

The present work was accomplished as a part of a larger multicentre health technology assessment project aimed at evaluating genetic testing for susceptibility to VTE. An exhaustive description of procedures and results and a deeper discussion in light of current literature will be reported elsewhere [manuscript in preparation]. For the purpose of the present manuscript, we will highlight a few elements that could be taken into account for further investigations.

In order to identify the most relevant questions and exploit them in a survey targeted to thrombophilia genetic testing, we inspected the ACCE model list of 44 questions (51). The three questions listed under the ELSI section (components: impediments; safeguards) were not relevant in the context under investigation, as we are not aware of discriminations or other legal and social issues related to genetic testing for VTE, neither of needs for specific safeguards. In fact, the ELSI element, as originally conceived, mostly covers population screening rather than individual tests and may be not adequately assessed in several instances (53). Therefore we explored the question related to the consequences of genetic testing, which was

<table>
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<th>(%)</th>
<th>Responders (n=97)</th>
<th>(%)</th>
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<td>1.3</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>Other indications</td>
<td>25</td>
<td>16.5</td>
<td>17</td>
<td>17.5</td>
</tr>
</tbody>
</table>

*Education level was ascertained by the means of the questionnaire and was not available for non-responders.
grouped under the clinical utility element. As it was already pointed out, the clients’ perspective pertains to great extent to the clinical utility of the specific genetic test under evaluation. The assessment of clinical utility in the ACCE paradigm should be strengthened, particularly by describing how and where genetic testing is used along the clinical path and by dissecting the different indications and clinical purposes of testing (52, 53). The clients’ survey reported here showed that thrombophilia genetic testing is currently ordered for a wide variety of clinical indications, ranging from a definite personal history of VTE to single unexplained pathological conditions. According to our findings, in some circumstances genetic testing is used as a screening tool in asymptomatic individuals (that is the case for assisted reproduction or prescription of oral contraceptives as indications for order). In this heterogeneous scenario, it appears quite difficult to estimate the actual impact of testing on clinical paths and on patients’ perception.

Our findings suggested that the delivery of test result does have a psychological impact, and that the perception of personal risk may change over time. Whether the genetic test result lead to relief of anxiety, or increased burden on relatives, for instance, influence the final health status. Keeping in mind that clinical utility could be phrased as the question: “is there an added value, in terms of health, in undergoing genetic testing?” evaluating clinical utility in susceptibility testing is firstly hampered by a methodological limitation. Dealing with susceptibility testing implies a prediction rather than an outcome, because diagnostic parameters cannot be observed at the time of testing. Therefore, the observation of clinical outcome requires data from longitudinal cohort studies, and cross-sectional designs are not appropriate. Furthermore, the psychological consequences of predictive genetic testing, that fit into overall clinical utility, should be followed-up in a long term, as they may change unexpectedly (54, 55).

In this regard, appropriate genetic counselling may help patients to appreciate the information provided by susceptibility testing, and to understand limitations as well clinical implications. However, our survey suggested that a minority of patients were referred to a clinical geneticist, though 28% of them (27/97) were tested due to family history or positive genetic test in relatives. Notably, a small percentage of non-carriers were referred to a genetic counselling visit (4.4%). Among 27 responders who were tested for positive family history (19+8, see Table 1), one underwent pre-test genetic counselling, three were suggested to undergo a visit with a clinical geneticist (2 carriers, 1 non-carrier; data not shown). It is noteworthy to underscore that genetic counselling should be suggested in carriers as well in non-carriers, if it is appropriate to help them (and their families) to appreciate how shared genetic and environmental factors underlie the recurrence of VTE in a family. It is quite obvious that also appropriateness of cascade testing in relatives may benefit from genetic counselling.

The study questionnaire indicated that the mere result of genetic testing, as extracted from the laboratory report, was adequately comprehended by almost all patients. Conversely, the clinical path after testing did not appear straightforward. More than one-third carriers did not receive any clinical prescription, at least in a short term, whereas a small proportion of non-carriers (7.4%) were prescribed a therapeutic intervention regardless of the negative test result.

In conclusion, our initiative contributed to highlight the need for additional studies aimed at evaluating the clinical utility of genetic testing in a real clinical context. Dedicated assessment procedures should develop accurate models incorporating genetic testing in the complexity of the individual clinical path. Using genomic information in a clinical meaningful way is

### Table 5.2

<table>
<thead>
<tr>
<th>Variant</th>
<th>Enrolled n(^a)</th>
<th>Carriers</th>
<th>%</th>
<th>Responders n(^a)</th>
<th>Carriers</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL (R506Q) heterozygous</td>
<td>143</td>
<td>13</td>
<td>9.1</td>
<td>92</td>
<td>8</td>
<td>8.7</td>
</tr>
<tr>
<td>PT (20210G&gt;A) heterozygous</td>
<td>121</td>
<td>13</td>
<td>10.7</td>
<td>82</td>
<td>11</td>
<td>13.4</td>
</tr>
<tr>
<td>MTHFR C677T homozygous</td>
<td>24</td>
<td>5</td>
<td>20.8</td>
<td>14</td>
<td>3</td>
<td>21.4</td>
</tr>
<tr>
<td>MTHFR C677T heterozygous</td>
<td>24</td>
<td>14</td>
<td>58.3</td>
<td>14</td>
<td>7</td>
<td>50.0</td>
</tr>
</tbody>
</table>

\(n^a\) refers to the number of individuals tested for each variant, respectively.

the challenge for personalised medicine. To accomplish this task, keeping the focus on the patients’ perspective is mandatory.

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VU is a PhD fellow at the University of L’Aquila, Italy.

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References


(2) De Stefano V, Za T, Ciminello A, et al. Epidemiology of venous thromboembolism and inherited thrombophilia in the western countries and in Italy. IJPH 2012; 9, Suppl. 1:S4.


(6) Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. A meta-analysis involving approximately 120 000 cases and 180 000 controls. Thromb Haemost. 2009; 102: 360-70


(11) INAHTA: HTA resources. Definitions, Available at http://www.inhta.org/HTA.

(12) CDC, ACCE Model, model, available on line at: http://www.cdc.gov/genomics/gtesting/ACCE/index.htm


(22) Bondy M, Mastromarino C. Ethical issues of genetic


(12) Recommendations from the EGAPP Working Group: routine testing for Factor V Leiden (R506Q) and prothrombin (20210G>A) mutations in adults with a history of idiopathic venous thromboembolism and their adult family members. Genet Med 2011; 13: 67-76


6. Testing for inherited thrombophilia: guidelines of the scientific societies

Valerio De Stefano, Tommaso Za, Angela Ciminello, Silvia Betti, Elena Rossi

6.1 INTRODUCTION

Venous thromboembolism (VTE) susceptibility genes are present in 5 to 10% of the general population and in at least 40% of patients with VTE (1, 2). An association with VTE has been firmly established for antithrombin (AT), protein C (PC), and protein S (PS) deficiency, as well as for factor V Leiden (FVL) and prothrombin (PT) 20210A (1-7). There is consistent evidence for a risk gradient for VTE, which is higher in carriers of AT, PC, PS deficiency and those homozygous or carriers of multiple defects, and moderate in heterozygous carriers of FVL or PT20210A (1-7). Accordingly, the search of the aforementioned inherited abnormalities is the only panel recommended for laboratory investigation of inherited thrombophilias (8-11). However, many experts consider testing for thrombophilia to be of little utility in the clinical management of the large majority of patients with VTE (5, 11-13). The association of inherited thrombophilia with arterial thrombosis or obstetric complications has been reported to be weaker and equivocal such that laboratory investigation in this setting is not warranted or should be conducted in selected patients (5, 10, 11, 14).

Despite such limitations, testing for inherited thrombophilia is common in clinical practice. A partial survey carried out in 2007 in Italy (60 million inhabitants) recorded about 22 000 tests for FVL and 20 000 tests for PT20210A (15). In 2007 in Australia (20 million inhabitants) 20 378 genetic tests for FVL were recorded (16). In current practice, the reason of testing for inherited thrombophilia is VTE in 42% of the checked patients, arterial thrombosis in 15-23%, and an obstetric complication in 13-17% (17, 18). Asymptomatic individuals account for 12-16% of testing because there is a known history of thrombophilia in a relative or there is a positive family history of VTE (16-18). Asymptomatic thrombophilia has been reported to have little impact on the risk for recurrence in two prospective studies (23, 24); as expected, in such investigations the most common gene polymorphisms associated with thrombophilia are FVL and PT20210A, present in nearly one third of the patients with VTE. Studies specifically aimed to investigate the risk for recurrence in carriers of either mutation gave conflicting results. The risk in a survey conducted in a tertiary hospital, 15% of the young women tested for FVL were referred before prescribing oral contraception (20).

6.2 TESTING FOR THROMBOPHILIA IN PATIENTS WITH VENOUS THROMBOEMBOLISM AND CONSEQUENCES FOR SECONDARY ANTITHROMBOTIC PROPHYLAXIS

After a first VTE the duration of secondary prophylaxis with oral anticoagulants (INR target 2 to 3) should be established weighing the risk for major hemorrhagic complications against the risk for a novel spontaneous VTE event. The risk of recurrent VTE is as high as 40% after 10 years from the first event (21), being low in patients having had VTE in association with circumstantial risk factors (surgery, trauma, pregnancy and puerperium, use of oral contraceptives) and maximal in patients with first spontaneous VTE (21-24). Prediction of recurrence should allow to select patients candidates to long-term (indefinite) duration of anticoagulation. Unfortunately the factors associated with a clinically relevant increase in risk for recurrence are not fully understood so far, being the final likelihood the resultant of clinical circumstances, features of early treatment, genotypes, laboratory global phenotypes (such as D-Dimer assay), and clinical global phenotypes (such as vein recanalization); the complexity of interactions and differences in study methodologies generates discrepancies of results and uncertainty in making decisions on thromboprophylaxis (25).

Inherited thrombophilia has been reported to have little impact on the risk for recurrence in two prospective studies (23, 24); as expected, in such investigations the most common gene polymorphisms associated with thrombophilia are FVL and PT20210A, present in nearly one third of the patients with VTE. Studies specifically aimed to investigate the risk for recurrence in carriers of either mutation gave conflicting results. The risk
for recurrent VTE among heterozygous carriers of either FVL or PT20210A has been recently revised by at least three meta-analyses (26-28). The former estimated that patients with first VTE and FVL or PT20210A have a 1.4-fold or 1.7-fold significant increase in the risk of recurrence, respectively (26). In a second meta-analysis restricted to prospective studies, the risk for recurrent VTE conferred by heterozygous FVL was 1.4-fold increased, whereas the risk found among heterozygotes for PT20210A was lower (27). A more recent systematic review found that heterozygosity for FVL was associated with a 1.6-fold increase in risk for recurrent VTE in probands, whereas heterozygosity for PT20210A was not predictive of recurrence (28). However the magnitude of the risk is modest and the hemorrhagic risk related with indication for long-term anticoagulation could be not justified in the majority of cases. In a prospective cohort of 599 patients with first VTE inherited thrombophilia was associated with a 1.8-fold increase in risk for recurrence; measurement of D-dimer levels was demonstrated to identify among patients with inherited thrombophilia a subset with low risk for recurrence (4.2% after 1.4 years of follow-up in the presence of normal D-dimer levels) and a subset with high risk for recurrence (27.1% in the presence of altered D-dimer levels) with a hazard ratio of 8.3-fold in comparison with the subset with low risk (29). Those findings give evidence that thrombophilia can not be considered as a whole and that further efforts are needed to clarify the role of mild thrombophilia in the interaction with other predictors of recurrent VTE and to identify subsets of patients at higher risk for recurrence.

Recent recommendations consider patients with AT, PC, or PS deficiency or multiple gene alterations not different from all the other patients with inherited thrombophilia as regards the duration of anticoagulant treatment (11, 12, 30). Yet it can be expected that in most studies the risk of recurrent VTE for the rare patients with deficiency of a natural anticoagulant is difficult to pick out since it is diluted by the weak effect of the much more frequent polymorphisms FVL and PT20210A. In a prospective cohort of unselected patients those with AT deficiency had a 2.6-fold increase in risk for recurrence, yet not significant likely for the low number of cases (23). In a retrospective controlled investigation we found that in the absence of anticoagulation AT deficiency is associated with a 1.9-fold significant increase in risk for recurrence in comparison with patients with no thrombophilia (31). Moreover in probands and their deficient relatives belonging to the EPCOT prospective cohort the incidence of recurrent VTE was 10.5 % patient-years in patients with AT deficiency and 3.5 % patient-years in carriers of FVL (32). In a retrospective investigation on proband patients with deficiency of natural anticoagulants and their deficient relatives the incidence of recurrent VTE was confirmed to be high, resulting 7.7 % patient-years (10 % for AT deficiency, 6% for PC deficiency, and 8.4% for PS deficiency) (33).

There is convincing evidence that patients with multiple defects are more prone to recurrent VTE (34-37). A retrospective study demonstrated that homozygotes for factor V Leiden show a higher risk for recurrent VTE than heterozygotes (38). In a systematic review homozygosity for FVL was estimated associated with a 2.6-fold increased risk for recurrent VTE (28). In conclusion, although the quality of the evidence in this area is low and does not allow firm recommendations, patients with AT deficiency, homozygosity for FVL, multiple defects, and perhaps PC or PS deficiency should be considered potential candidates for long-term oral anticoagulation after a first unprovoked VTE. This has been accepted by an International Consensus Statement in 2005 (9) and, more recently, by the French consensus guideline on testing for thrombophilia in VTE (19). It should be underlined that the conditions above listed are present in a not negligible portion of patients with VTE, being identifiable in at least 10% of them. Nevertheless, American and British guidelines consider routine testing not justified among patients with VTE (11-13). The recommendations of the published guidelines of scientific societies or international working groups are summarized in Table 6.1.

A special situation is the occurrence of rare thromboses in the unusual sites such as cerebral or splanchnic veins; in this setting up to half of the patients carry inherited thrombophilia (39). The optimal duration of anticoagulant treatment after a first event is unknown, but international guidelines recommend indefinite anticoagulation in the presence of persistent risk factors (e.g. thrombophilia) for patients with cerebral vein thrombosis (40) or patients with extrahepatic portal vein obstruction (41), so that laboratory investigation is warranted. On the other hand, British guidelines on inherited thrombophilia warn that decisions regarding duration of anticoagulant
### Table 6.1

<table>
<thead>
<tr>
<th>Guideline Description</th>
<th>Value of testing for knowledge of reason of VTE</th>
<th>Value of testing for prediction of recurrence after unprovoked VTE</th>
<th>Value of testing for prediction of VTE and prescription of antithrombotic prophylaxis in asymptomatic relatives</th>
<th>Value of testing for prediction of VTE and prescription of antithrombotic prophylaxis in the general population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International Consensus Statement, 2005</strong> (9)</td>
<td>Yes, in all patients (except those with a single provoked VTE &gt; 50 years)</td>
<td>Yes (deficiency of AT, PC, PS, homozygotes, double heterozygotes for FVL and PT20210A)</td>
<td>Yes (in particular females in fertile age)</td>
<td>No</td>
</tr>
<tr>
<td><strong>American College of Chest Physicians (ACCP) Guidelines, 2008</strong> (12)</td>
<td>Not analyzed</td>
<td>Potentially useful in selected patients as part of an overall risk/benefit evaluation of indefinite anticoagulation</td>
<td>Not analyzed</td>
<td>Not analyzed</td>
</tr>
<tr>
<td><strong>French Consensus Guideline, 2009</strong> (19)</td>
<td>Yes, in patients with a single unprovoked proximal DVT and/or PE &lt; 60 year, in patients with recurrent proximal DVT and/or PE, and in patients with recurrent unprovoked distal DVT &lt; 60 years)</td>
<td>Yes (deficiency of AT, PC, PS, homozygotes, double heterozygotes for FVL and PT20210A)</td>
<td>Yes (possible exception for relatives of probands isolated heterozygotes for FVL and PT20210A)</td>
<td>No</td>
</tr>
<tr>
<td><strong>British Committee for Standards in Haematology, 2010</strong> (11)</td>
<td>No (possible exception for those with a strong family history of unprovoked recurrent VTE)</td>
<td>No</td>
<td>No (possible exception for relatives of probands with deficiency of AT, PC, PS)</td>
<td>No</td>
</tr>
<tr>
<td><strong>Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group, 2011</strong> (13)</td>
<td>No (analysis limited to FVL and PT20210A)</td>
<td>No (analysis limited to FVL and PT20210A)</td>
<td>No (analysis limited to FVL and PT20210A)</td>
<td>Not analyzed</td>
</tr>
</tbody>
</table>

VTE: venous thromboembolism; DVT: deep venous thrombosis; AT: antithrombin; PC: protein C; PS: protein S; FVL: factor V Leiden; PT20210A: prothrombin 20210A
therapy in relation to the results of testing are not evidence-based (11).

Pregnant women with a previous history of unprovoked or estrogen-related or pregnancy-related VTE should be offered antenatal antithrombotic prophylaxis independently on the presence of thrombophilia. Women with a previous VTE provoked by a major transient risk factor such as surgery or major trauma would not usually require prophylaxis (10, 11, 42). Laboratory investigation for thrombophilia is warranted in women with a previous VTE provoked by a minor risk factor, because as this will influence management and decisions regarding thromboprophylaxis antenatally (11, 42).

6.3 TESTING FOR THROMBOPHILIA IN ASYMPTOMATIC INDIVIDUALS AND CONSEQUENCES FOR PRIMARY ANTITHROMBOTIC PROPHYLAXIS

VTE is a common complex disease, being the resultant of gene-gene and gene-environment interaction. Unfortunately, a simple model due to the presence or the absence of two dichotomous factors (high-risk allele and exposure to an environmental risk factor) is not reliable in most of the cases. This is due to incomplete clinical penetrance of genotypes, since not all carriers develop VTE during life, and to variable expressivity of severity and age of onset of the disease. Moreover, the onset of disease is modulated also by gene-gene interactions, in the large majority of cases still obscure, and by multiple effects of various environmental risk factors, acting on the genotype by additive or multiplicative way. The above limitations render so far of little or null clinical utility indiscriminate genetic testing of populations for VTE-susceptibility genes and unlikely to compete for resources with other medical interventions (43). Universal screening before exposure to environmental risk circumstances such as oral contraceptive intake or pregnancy has been estimated not cost-effective too (43-45). Moreover, individuals labeled as carriers by random screening could experience insurance discrimination or feel undue anxiety receiving no real benefit in terms of prevention. In conclusion, general population screening is discouraged because of doubtful utility and potential detrimental effect on the carriers (46-48).

Targeted screening in the siblings of the index patients with VTE is obviously more fruitful than in the general population, with a diagnostic yield of 50%, being such traits genetically dominant. The primary argument for screening asymptomatic relatives of patients with thrombophilia is the possibility of reducing the occurrence of provoked VTE, by offering advice concerning primary antithrombotic prevention during circumstances that could potentially lead to VTE and that are not usually covered with prophylaxis (e.g. low-risk surgery or pregnancy and puerperium), and counseling carrier women about the use of hormone therapies. In all guidelines, special attention is paid to asymptomatic women of childbearing age, especially in the presence of a family history of VTE and/or a familial AT, PC, PS deficiency, homozygous FVL or PT20210A or double heterozygous FVL and PT20210A (9, 10, 11, 42).

However, this type of counseling should be weighed against potential detrimental effects in the carriers, such as emotional burden due to an overestimated perception of risk (49-53). The presence of a family history of VTE may be a way to engage in the targeted case-finding of carrier relatives who may be at higher risk. In fact, family history of VTE has been consistently reported to be a risk factor for VTE independent of the presence of known thrombophilic abnormalities (54-58). Moreover, the carriers of thrombophilia with a family history of VTE have been reported to be more prone to VTE than those without (56, 59, 60).

Several family studies have investigated the risk for VTE among relatives of individuals with inherited thrombophilia (reviewed in ref. 60). In both prospective and retrospective studies, the incidence of VTE among relatives was higher in carriers of AT, PC, or PS deficiency, with a range between 0.36 and 4.0 % individual-years. The highest incidence was consistently observed among carriers of AT deficiency, with 1.0 to 4.0 % individual-years. In studies using unaffected relatives as the reference group, the risk for VTE among carriers of AT, PC, or PS deficiency was 4 to 30 times greater than that in non-carriers. On the other hand, a lower incidence of VTE was reported among the relative carriers of FVL and PT20210A, consisting of 0.19 to 0.58 % individual-years for FVL, and between 0.11 and 0.37 % individual-years for PT20210A. The low absolute incidence of VTE reported in relatives of patients with FVL or PT20210A has prompted many experts to consider familial screening for inherited thrombophilia to be unwarranted in this setting, as it is without high clinical utility (49,
REFERENCES

(20) Gartner V, Weber M, Eichinger S. The emotional impact of...
of genetic testing and aspects of counseling prior to prescription of oral contraceptives. Contraception 2008; 78: 392-8


(39) Martinelli I, De Stefano V. Rare thromboses of cerebral, splanchnic and upper-extremity veins. A narrative review. Thromb Haemost 2010; 103: 1136-44


(42) Royal College of Obstetricians and Gynaecologists. Reducing the risk of thrombosis and embolism during pregnancy and the puerperium. Green-top Guideline No. 37, 2009; available on line at: www.rcog.org.uk (last access: 20 November 2011)


(49) Green D. Genetic hypercoagulability: screening should be an informed choice. Blood 2001; 98: 20
(60) Rossi E, Ciminello A, Za T, et al. In families with inherited thrombophilia the risk of venous thromboembolism is dependent on the clinical phenotype of the proband. Thromb Haemost 2011; 106: 646-54
The purpose of our work was to carry out a Health Technology Assessment project on genetic testing for susceptibility to Venous Thromboembolism (VTE) in Italy. Our experience was carried out using the ACCE model, developed by the National Office of Public Health Genomics, CDC, for the evaluation of genetic tests. It aims at providing a complete summary of all the available information that may be useful to policy-makers, health professionals and consumers. This ACCE framework has been applied to genetic test evaluation for single-gene disorders by the Genetic Testing Network in the UK and by the Evaluation of Genomic Applications for Practice and Prevention (EGAPP) project in the USA. Venous thromboembolism (VTE) is an extremely common medical problem manifested as either deep venous thrombosis (DVT) or pulmonary embolism (PE) affecting apparently healthy as well as hospitalized patients. VTE is one of the leading causes of mortality and morbidity in the developed world. Prothrombin (PT) 20210A, factor V Leiden (FVL) G1691A and ethylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms are the most common inherited risk factors for VTE. In Italy the epidemiology of VTE and the polymorphisms above mentioned was investigated by the Vicenza Thrombophilia and Atherosclerosis (VITA) Project that collected clinical data and a blood sample from 15 055 individuals aged 18-65. Results were as follows:

- The prevalence of VTE was 7.7 per 1 000 inhabitants.
- The prevalence of factor V Leiden was found 2.4% for heterozygotes and 0.1% for homozygotes.
- The heterozygosity for prothrombin G20210A was found in 4.3% of the cases with VTE and 3.4% of the population without VTE; no homozygous carrier was found.
- The prevalence of homozygotes for the MTHFR C677T polymorphism was 12.3% among the patients with VTE and 13.8% among the controls.

In the sketch of the real-world practice, it was confirmed that genetic testing for thrombophilia is widely offered by Italian laboratories. There is a need for shared quality assessment procedures and for effective dissemination of evidence-based recommendations.

Our results indicate a high analytic validity for both FVL and PT20210A. Also the data reported in the literature most laboratories can test for FVL and PT20210A with a high degree of reliability. Multiple SNPs analysis by microarray provides a remarkably wider piece of genetic information, which can be used as a better predictor for diseases occurrence.

Concerning the clinical validity, our study showed that FVL is associated with the highest risk of developing VTE (overall OR: 3.68; 95% CI: 2.83-4.52). As expected, women assuming OCs resulted as the population with the highest risk of developing VTE (women assuming OCs, OR: 7.82; CI: 3.793-16.11; women not assuming OCs, OR: 3.46; CI: 2.40-5.01). PT20210A resulted associated with an increased risk of developing VTE (overall OR 2.12; CI: 1.77-2.47), albeit not as strongly as FVL. As for MTHFR, the overall analysis (OR: 1.14; CI: 0.76-1.52) and the stratified analyses indicated that the MTHFR variant is not associated with a significantly increased risk of developing a VTE. Additionally, presented results provide evidence that the analysis of single SNPs for prothrombin II and V may be predictive for thromboembolism occurrence only for homozygous mutant, which are very rare in the population, and mainly at younger (<54 years) age. At older ages environmental exposures and lifestyle factors overwhelm the role of genetic risk factors. Single SNP analysis as currently performed in clinical practice makes available only a very small piece of information as compared to the whole individual genetic asset. Multiple SNPs analysis by microarray provides a remarkably wider piece of genetic information, which can be used as a better predictor for diseases occurrence.

For clinical utility, eight guidelines on genetic tests for VTE included, produced or updated from 2003 to 2010 were evaluated. According to these clinical recommendations, intercurrent events play an important role in increasing the risk in patients with an established presence of genetic thrombophilic mutations. These events include: recurrent VTE, pregnancy,
use of oral contraceptives, surgery and travels that provide a period of prolonged immobilization (> 8h). In these cases, the recommendations of the guidelines are indications for prophylaxis with a preventive treatment.

As far as concerns the **economic aspects**, although the results of the considered studies are not always comparable and in agreement, genetic screening for VTE appears to be cost-effective only for targeted populations who are already at risk (with a personal or familial history of VTE), especially when the risk is augmented further by specific conditions such as pregnancy, the use of oral contraceptives, or surgery.

The **ethical evaluation** founded on the value and centrality of the human being, showed that it is important not only to help people understand the differences between mutation and disease, risk assessment, susceptibility penetrance, polygenicity, the interaction between genes and environment, the possibility of false negatives and false positives in genetic testing, but also to help people make choices responsibly. For this reason, education should focus on scientific facts, but it should also encompass psychological, social and ethical aspects. The education of patients lies in the hands of family physicians, who should act as intermediaries between the patients and the genetic services.

Genetic testing for thrombophilia did not appear to be effectively incorporated into standard clinical path. Our investigation of the real-world practice underscored the need for additional studies aimed at assessing the clinical utility of genetic testing for susceptibility to common disorders. Educational programmes for health professionals may be helpful.

In conclusion, although the quality of the evidence in this area is low and does not allow firm recommendations, patients with AT deficiency, homozygosity for FVL, multiple defects, and perhaps PC or PS deficiency should be considered potential candidates for long-term oral anticoagulation after a first unprovoked VTE. This has been accepted by an International Consensus Statement in 2005 and, more recently, by the French consensus guideline on testing for thrombophilia in VTE. It should be underlined that the conditions above listed are present in a not negligible portion of patients with VTE, being identifiable in at least 10% of them. Nevertheless, American and British guidelines consider routine testing not justified among patients with VTE. Some guidelines consider justified familial screening only for relatives of probands with AT, PC, or PS deficiency or probands with multiple abnormalities. However, it should be kept in mind that among the relatives of probands isolated heterozygotes for FVL and PT20210A, some asymptomatic individuals could be carriers of multiple abnormalities and, therefore, could receive a benefit from diagnosis.

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