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Contribution of Molecular Biology in the Assessment of Thrombophilia

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Abstract

The term thrombophilia designates, on the one hand, clinical situations characterized by the occurrence of early or recurrent venous thrombosis or an unusual site, on the other hand, biological situations characterized by hypercoagulability. The objective of our study is to assess the role of molecular biology in the assessment of thrombophilia, in particular for the identification of the deficiency or molecular abnormality of coagulation factors responsible for the thrombotic tendency. The present work is a retrospective descriptive and analytical cross-sectional study, carried out in the biological hematology department of the Avicenna military hospital, over a period of 3 years. We searched by PCR technique for the factor V Leiden mutation and the factor II G20210 mutation in all patients hospitalized for an etiologic diagnosis of constitutional thrombophilia. Of the 30 patients included in our study, 18 were women and 12 men. The average age was 42. 77% of patients were over 45 years old and only 23% were under 45 years old. The internal medicine service was responsible for 50% of requests, followed by external requests (23%) for various indications, mainly related to deep vein thrombosis (56.67 %), pulmonary embolism (30%). Regarding the results of the PCR, the Factor V Leiden mutation was found in a single patient, and the Factor II G20210 mutation was found in 3 patients. The existence of thrombophilic constitutional anomalies is today accessible to biological diagnosis in a codified and reliable manner. While the indication for this specialized thrombosis assessment is well established, the results obtained are nevertheless decisive and will further reduce the percentage of unexplained thromboembolic disease and ensure better targeted prophylaxis.

Keywords: Factor V Leiden mutation; Factor II G20210 mutation; Molecular biology; Thrombophilia.

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1. Introduction

The term "thrombophilia" designates on the one hand a clinical situation characterized by a tendency to thrombosis, and on the other hand biological abnormalities predisposing to thrombosis; It is of multifactorial origin and heterogeneous clinical expression [1]. On the biological level, this term designates, according to the HAS, the anomalies or peculiarities of coagulation, identifiable by laboratory tests, which predispose to venous thromboembolic disease. These abnormalities or peculiarities, or even biological risk factors for Venous Thromboembolic Disease (VTE), can be hereditary (genetic) or acquired [2]. Genetically, thrombophilia may be due in the first place to a deficiency in coagulation inhibitors such as: antithrombin, protein C or protein S. Secondly, abnormalities such as hyperhomocysteinemia, an excess in factor are noted. VIII, resistance to activated protein C (most often linked to the Factor V Leiden mutation) and the Factor II mutation [3]. These last two mutations are the subject of our study. The factor II or prothrombin (G20210A) mutation relates to the replacement of G by A at nucleotide 20210 in the 3 'untranslated region of the gene and is associated with increased plasma prothrombin levels [4]. Factor V Leiden (G1691A) refers to the replacement of G by A at the nucleotide at position 1691 of the factor V gene, resulting in the substitution of arginine, amino acid, by glutamine in the factor V protein, and causing resistance to cleavage by activated protein C (PCA) [5]. The association of factor II (G20210A) and factor V Leiden (G1691A) mutations with an increased risk of venous thrombosis has been well documented. Obviously, the search for these constitutional anomalies should not obscure the need to search for acquired pathologies with more important therapeutic consequences such as anti-phospholipid syndrome or the presence of cancer. The objective of our study is to show the interest of molecular biology in the assessment of thrombophilia as part of the diagnostic management of unexplained thrombosis.

2. Materials and methods

2.1. Type of study

It is a retrospective, descriptive, analytical study spread over a period of 2 years, going from May 2018 to June 2020. It was interested in the 30 requests for genetic study within the framework of thrombophilia assessment received by the Department of Biological hematology from the various departments of the Avicenne Military Hospital, the MOHAMMED VI CHU and outpatient clinics.

2.2. Study population

- ***Inclusion criteria***

We included in our study patients hospitalized in the various departments as well as patients seen in outpatient consultations who presented a venous thrombotic episode (deep vein thrombosis or pulmonary embolism), unexplained arterial thrombosis, recurrent miscarriages, patients with risk (pregnancy with a history of venous thrombosis) and for which the request for a genetic study as part of the thrombophilia assessment was carried out at the central hematology laboratory of the AVICENNE military hospital (HMA) in Marrakech.

- ***Exclusion criteria***

All patients with incomplete information were excluded from our study.

2.3. Methods

- ***Data collection***

To conduct this study, an operating sheet was produced to collect information from the archive registers of the HMA hematology laboratory.

- ***Assay methods***

-The pre-analytical phase: The steps of the pre-analytical phase have been respected. Samples were taken from fasting patients, away from any heparin therapy, on silicone glass EDTA tubes, with the tourniquet moderately tight and held for less than one minute. The transport conditions were within the standards with a test completion time of less than 4 hours.

- The analytical phase: The detection of these two mutations is carried out by the real-time PCR technique on a GeneXpert system which consists of an instrument, a personal computer, a portable barcode reader and preloaded software for running tests and viewing results. This GeneXpert Dx system automates and integrates sample purification, nucleic acid amplification and target sequence detection in whole blood. The system requires the use of disposable single-use cartridges that contain the PCR reagents and house the PCR process. Cross-contamination between samples is eliminated because the cartridges are independent. The Xpert Factor II & Factor V assay includes reagents for the detection of normal and mutant factor II and factor V alleles. The primers and probes in the Xpert Factor II & Factor V assay determine the genotype of the factor II gene (at position 20210) and / or the factor V gene (at position 1691).

-Post-analytical phase: The results are automatically interpreted by the GeneXpert DX system from measured fluorescent signals and built-in algorithms to identify genotypes, then are displayed in the "View results" windows. Then printed and made available to specifiers.

- ***Statistics***

The data was entered and processed using SPSS 25.0 and Excel 2019 softwares.

- ***Ethical aspects***

After approval by the ethics committee, patient’s consent was obtained.

Funding of this study was from strictly institutional sources.

Respect for anonymity and confidentiality were taken into account during Data collection.

3. Results

Of the 30 patients included in our study, 18 were women and 12 men, for a sex ratio of 0.6. The average age was 42 with extremes ranging from 19 to 53. The peak incidence is between 40 and 50 years. 77% of patients were over 45 years old and only 23% were under 45 years old. The internal medicine department was responsible for 50% of requests (50%), followed by external requests (23%) and the cardiology service (13%) (Table I) for various indications, mainly due to deep vein thrombosis (56.67%), pulmonary embolism (30%), ischemic stroke (6.7%), Recurrent miscarriages (3.3%), Cerebral thrombophlebitis (3.3%).

Regarding the results of the PCR, among the 30 requests received, the Factor V Leiden mutation was found in a single patient, i.e. a percentage of 3.33%, and the Factor II G20210 mutation was found in 3 patients, i.e. a percentage of 10%.

Apart from PCR, various tests were carried out. The assessment combining TP, TCA and CBC was requested for all patients, followed by the assessment combining the assay of protein C, antithrombin, and protein S (Figure 1).

Table 1: breakdown of balance sheets according to requesting services.

Department	Number	Percentage
internal Medicine	15	50,00%
external	7	23,33%
cardiology	4	13,33%
Intensive care	1	3,33%
hematology CHU	1	3,33%
neurology CHU	1	3,33%
gynecology CHU	1	3,33%

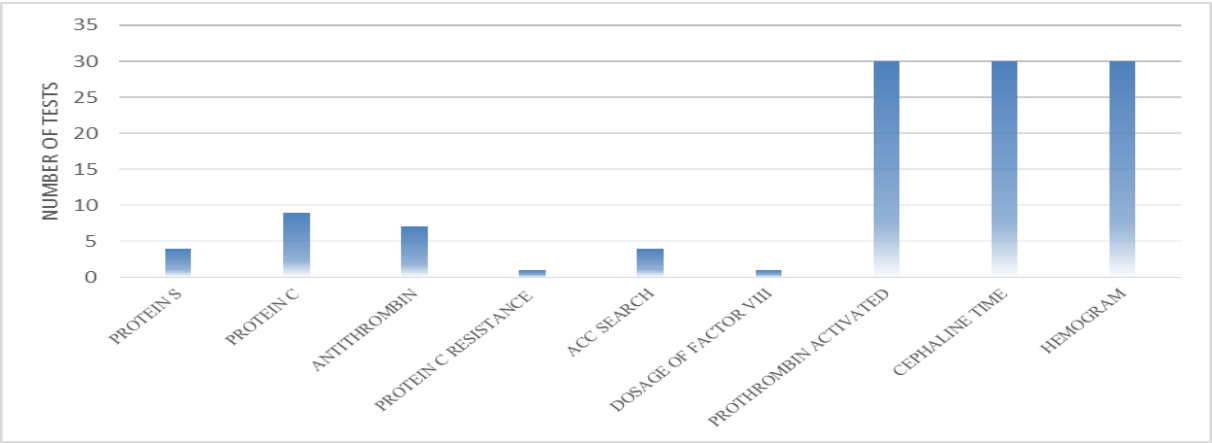

Figure 1: Other tests carried out**.**

4. Discussion

Venous thrombosis is a major medical problem affecting millions of people around the world every year. The mutation in the factor V (FV) gene is the most common genetic risk factor known to date. Another relatively common risk factor is a point mutation in the prothrombin gene. Combinations of genetic defects are relatively common in the general population. [1]

Leyden's FV (FVL) is a point mutation characterized by the replacement of nucleotide 1691 G by an A in exon X of the FV gene, which is responsible for the replacement of Arg-506 by a glutamine [6]. This polymorphism modifies the predominant cleavage site of FVa by Activated C Protein. This causes the phenomenon of resistance of the plasma to the action of Activated C Protein [7]. DNA testing for the G / A 1691 mutation is easily performed using conventional molecular biology techniques [6].

Factor V Leiden, heterozygous, is present in 2-5% of subjects of European (Caucasian) descent, which is consistent with the results of our study. It varies from region to region and reaches 9% in Alsace and 10 to 15% in certain regions of Sweden and Cyprus [7]..

In heterozygous subjects, the risk of venous thrombosis is increased by about 5 to 7. In many cases, heterozygous subjects are asymptomatic. Venous thrombosis can occur in risky situations, which act as a triggering factor, such as surgery, pregnancy, taking oral contraceptives, immobilization. For example, the combination of oral contraception and heterozygous factor V Leiden could lead to a 50-fold increase in the risk of venous thrombosis. After a first thrombosis, the risk of recurrence is increased, and could reach 50%. In subjects homozygous for factor V Leiden, the risk of venous thrombosis seems high (risk multiplied by 20), and asymptomatic subjects are rarer. Risky circumstances are particularly poorly tolerated [8].

The prevalence of the Leiden mutation in deep vein thrombosis (DVT) and pulmonary embolism (PE) has been studied by different teams who have found more or less consistent results, some showing a lower frequency of this mutation in PE and severe DVTs and others showing no relationship between the Leiden mutation and the degree of severity of the venous thromboembolic disease [3]. Furthermore, some studies showed that Factor V Leiden presents not a major role as risk factor for arterial thrombosis, while it is present in 18% of Caucasian patients with venous thrombosis. This high incidence prevalence mirrors the incidence in the corresponding general populations and can be even higher in some areas according to the ethnic background [7].

Prothrombin is a precursor of vitamin K dependent thrombin, the terminal enzyme of the coagulation cascade. A single nucleotide mutation in one of the prothrombin genes at position 20210 results in the presence of elevated levels of plasma prothrombin and increases the risk of venous thrombosis. The prevalence of Prothrombin G20210A mutation in European Caucasians was found to be roughly 3–17% in patients with VTE and 1–8% in healthy controls. That was also true in Caucasians living outside Europe like in the USA, Australia, Brazil and Israel [9, 10], which is consistent with the results of our study (10%). On the other hand, Prothrombin G20210A mutation was found to be very rare or even absent in Asian and African populations, and in native populations of America (Amerindians) and Australia. More studies with bigger samples are needed in our country and in the

African country to have precise data on our own epidemiology.

Other studies have shown that the prevalence of the 20210A allele is indeed 1 to 2% in the normal population. On the other hand, in subjects with a tendency to thrombosis, the prevalence is of the order of 5 to 7%, according to the most recent studies, and therefore slightly lower than initially reported. The relative risk of venous thrombosis would be increased by 3 to 5 times, and would be higher in subjects who present at the same time a factor V Leiden [11].

5. Conclusion

Genetic analysis of inherited thrombophilias has matured in recent years, and understanding of the genetics underlying the disorders has increased. The pathophysiological aspects of constitutional thrombophilia are attractive and constitute a very dynamic field of research. However, these abnormalities are rare and their relatively modest therapeutic implications when considered as a group of patients with venous thromboemobolic disease. They should therefore in no way mask the importance, in an etiological assessment, of looking for other more frequent contributing factors with far greater therapeutic consequences.

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6. Competing interests

• The authors declare that they have no competing interest