

Counterpoint

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The futility of thrombophilia testing

Abstract: There has been increasing recognition of various laboratory markers of thrombophilia that are associated with increased risk of thrombosis either through hereditary (especially Factor V Leiden, prothrombin G20210A mutation, and protein C, S and antithrombin deficiencies) and/or acquired means (e.g., antiphospholipid antibodies) over past decades. This has led to an explosion of clinical requests for these markers, that has now become virtually uncontrolled, and seemingly inclusive of everyone who has had a thrombotic event. Although these haemostasis-related defects should be assessed in selective cases, the overuse (or misuse) of testing causes serious adverse outcomes and leads to the conclusion that, in general, testing for thrombophilia is futile.

Keywords: acquired thrombophilia; antiphospholipid antibodies; antithrombin; Factor V Leiden; inherited thrombophilia; protein C; protein S; prothrombin mutation; testing.

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The history of the study of thrombophilia is interesting and reviewed elsewhere [1, 2]. The initial major discoveries for inherited thrombophilia were for deficiencies in protein C (PC), protein S (PS) and antithrombin (AT), which were later supplemented by identification of the gain-of-function mutations Factor V Leiden (FVL) and prothrombin G20210A (PGA). The inherited PC/PS/AT deficiencies are very rare (<0.5%) in the general population but are associated with a more severe thrombophilic tendency, whereas the FVL/PGA mutations are more common (approx. 5%) in the general population but associated with a less severe thrombophilic tendency. The acquired thrombophilia marker, antiphospholipid antibodies, has a different history but is similarly associated

with an increased risk of thrombosis, as well as pregnancy morbidity/mortality [3].

Each of these conditions is present in only subsets of individuals who have had a thrombosis. For example, PC/PS/AT deficiencies would be identified in probably <5% of unselected cases of venous thromboembolism (VTE). In contrast, by virtue of being more common in the general population, FVL/PGA mutations would expectedly be identified in a larger proportion (perhaps approaching 50% of unselected VTE cases). There are other noted differences. For example, whereas VTE associated with PC/PS/AT deficiencies tends to occur at a young age (<50 years), VTE associated with FVL/PGA mutations may occur later and be additionally associated with other acquired risk factors (e.g., surgery, trauma, pregnancy).

Although assessment for each of these conditions may be useful in select cases, there are many reasons why the general assessment of thrombophilia in patients is not useful, even in those who have had a thrombosis. Indeed, 'less discriminate' testing leads to outcomes that are worse than not having investigated at all. Unfortunately, this ill-fated situation appears to be the new reality, and the clinical recognition that there are thrombophilia biomarkers available for ordering has translated to an uncontrolled tendency to request these tests on virtually all patients presenting with a thrombosis. The danger of this approach is summarised in Table 1.

PC/PC/AT

As mentioned, PC/PC/AT are rare in the general community (<0.5%) and will at best only be identified at a rate of approximately 5% in unselected VTE. Laboratory normal reference ranges (NRR) are most typically established using 95% confidence intervals, meaning that they will 'confidently capture' 95% of the normal population. However, what this also means is that 5% of the normal population will (by definition of the NRR process) fall outside these limits; thus, approximately 2% of normal individuals will be identified as falsely having a PC or

Table 1 Summary of problems associated with thrombophilia testing.

Thrombophilia marker(s)	Problem(s)	Adverse outcome(s)
PC/PS/AT deficiencies	1. Low incidence in normal population (<0.5%) and unselected VTE population (~5%). 2. 'Consumed' by the thrombosis event and also affected by anticoagulant therapy as typically applied to all patients who have a thrombosis. 3. NRR effect: ~2% false-positive rate for each test; cumulative potential for ~6% false positive for any single marker (PC, PS or AT). 4. Clinicians are not appropriately selecting patients for investigation.	1. High risk for potential false positives that exceeds likely true-positive rate by factor of 2–10×. 2. Potential life-long label of 'false' PC/PS/AT deficiency, or else lingering residual of this 'diagnosis' in LIS or medical records. 3. Requirement to reverse false label of PC/PS/AT deficiency by retesting and patient counselling. 4. Identification of true deficiencies have moderate clinical utility and will in many cases not affect the individual's clinical management. 5. Risk of patient over-treatment based on laboratory finding.
FVL/PGA mutations	1. Relative high incidence in normal population (~5%) and unselected VTE population (up to 50%). 2. Clinicians are not appropriately selecting patients for investigation.	1. High chance of identifying these mutations 2. Identification of mutation has low clinical utility and will in general not affect the individual's clinical management 3. Risk of patient over-treatment based on laboratory finding. 4. Psychological and insurance implications from being identified to have a genetic mutation that increases thrombotic risk.
aPL	1. Relative high incidence in normal (asymptomatic) population (up to 5%). 2. Clinicians are not appropriately selecting patients for investigation. 3. Some tests affected by anticoagulant therapy (as typically applied to all patients who have a thrombosis); thus false positive and negatives possible. 4. Some tests have high inter-laboratory variability and thus low clinical utility.	1. Possible risk of misdiagnosis 2. Possible risk of under or over-treatment of patient based on 'false'-negative or -positive laboratory finding. 3. Possible risk of over-treatment of asymptomatic aPL positive patient.
General		1. Patient anxiety and family issues for 'hereditary' disorders. 2. Expensive and doubtful cost benefit, especially when testing inappropriately applied. 3. Presence or absence of thrombophilia marker usually does not change clinical or therapeutic management. 4. Lack of thrombophilia marker will not prevent a potential thrombosis and presence of thrombophilia marker does not guarantee a future thrombosis.

aPL, antiphospholipid antibodies; AT, antithrombin; FVL, factor V Leiden; LIS, laboratory information system; NRR, normal reference range; PC, protein C; PGA, prothrombin G20210A; PS, protein S; VTE, venous thromboembolism.

PC or AT deficiency with each round of testing for each parameter. As these 'false low values' may identify different normal individuals for each of the three different tests evaluated, upwards of 6% of normal individuals may

in total therefore be falsely identified as having a PC or PC or AT deficiency following testing of all three parameters. Thus, the risk of falsely being identified as PC or PC or AT deficient (approx. 6%) is similar to the chance

of identifying a true deficiency in unselected VTE cases (approx. 5%), and $>10\times$ the chance of identifying a true deficiency in a normal population ($<0.5\%$). A second consideration is the timing of the clinical request or blood sampling. Reductions in PC/PC/AT levels may occur just after a thrombosis due to ‘consumption’ or inflammatory events, and thus testing patients at this time can lead to detection of additional false deficiencies. Moreover, patients having suffered a thrombosis such as a VTE are subsequently placed on anticoagulant therapy, to both treat the thrombosis and prevent extension or reoccurrence of thrombosis, and potentially including unfractionated heparin, low molecular weight heparin, vitamin K antagonists such as warfarin, or perhaps one of the newer oral anticoagulants such as rivaroxaban [4, 5]. Each of these anticoagulants will variably affect PC/PC/AT assays [6, 7], and samples taken from patients once commenced on these drugs have a very high chance of yielding a false deficiency. We also know from audits of clinical ordering practice that between 30% and 50% of test requests for PC/PC/AT assays occur while patients are on anticoagulant therapy [8, 9]. Indeed, one recent audit revealed that an alarming 80% of test cases determined to have low PC or PC or AT were likely to have derived from patients on anticoagulant therapy, and thus were potentially false-positive events [9].

FVL/PGA

As mentioned, FVL/PGA are not so rare in the general community (approx. 5% in whites). Compared to PC/PS/AT, there is much less chance of a ‘false-positive’ finding since genetic tests tend to provide more definitive yes/no answers. However, the relatively common presentation of FVL/PGA in the general population means that these can ‘easily’ be found in both thrombosis affected and unaffected individuals. Although FVL/PGA increase an individual’s risk of thrombosis, particularly when they coexist with other identifiable acquired risk factors, there is a clear danger that both clinicians and patients will overinterpret the importance of detection of FVL/PGA mutations. When clinicians become less selective of patients to investigate, the FVL/PGA identified becomes increasing irrelevant, with total irrelevance achieved when detection rates approach those of the normal population, since in essence this means that the normal population is being tested for these thrombophilia markers. Recent audits of clinical ordering and test practice have indeed confirmed this to be the case [9, 10] (Figure 1). Moreover, the same audits have revealed that current clinical requests are simply following other natural event trends, namely births by age for women, and age-related VTE rates for males; thus, the current clinical ordering patterns simply

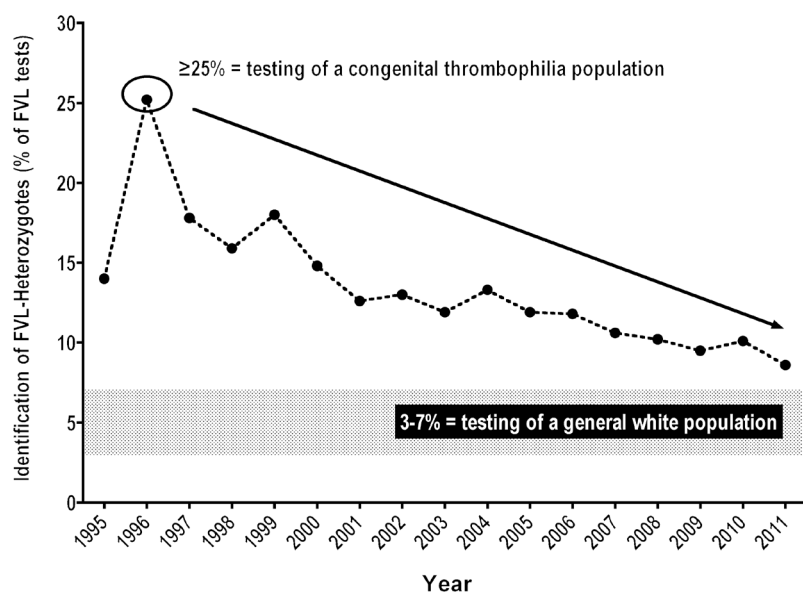


Figure 1 Decreasing rate of FVL heterozygote detection (as a percentage of FVL tests performed) during the past 17 years at the author’s institution (modified from [9, 10]).

appear to be following pregnancy trends in women and VTE occurrence trends in males.

Antiphospholipid antibodies

These can be identified by either clot-based assays that detect so-called lupus anticoagulants (LA) or solid phase assays that detect other antiphospholipid antibodies (aPL) [6, 11]. The anticoagulants mentioned previously, given as thrombosis therapy and affecting PC/PS/AT assays, also affect the clot-based assays used to identify LA, and can differentially produce both false-positive and false-negative results [6]. Although solid phase assays aPL assays are generally unaffected by these anticoagulants, they are instead biased by much higher inter-laboratory variation than LA testing [12, 13], thereby limiting their clinical utility. In summary, some laboratories will report an aPL negative sample as being aPL positive, some laboratories will report an aPL positive sample as being aPL negative, and the strength of an aPL positive sample will also be variably reported. Finally, evidence that clinicians are inappropriately ordering aPL testing is also available. As an example, despite its proven association with pregnancy morbidity/mortality, one recent audit identified that none of the 72 consecutive obstetric patients tested at one institution within a 6-month period was identified to have aPL [14]. The conclusion here is that the presence of aPL is either uncommon in the obstetric patient group (which we know to be untrue), or that the clinical reasoning for laboratory investigation of aPL by the obstetrics team require refinement, and specifically better patient selection.

In summary, the current evidence indicates that appropriate patient selection for thrombophilia investigation is simply not occurring, and instead clinicians are requesting such tests fairly unselectively. This has several adverse consequences (Table 1). First, it is costly and wasteful of health resources. Second, it is important to remember that identification of a ‘false positive’, be it PC, PC, or AT deficiency in an anticoagulated patient, or a FVL or PGA mutation in a 50-year-old woman without any additional risk factors, do not represent benign discoveries. Significant adverse effects will arise from these events, including the patient’s psychological distress at discovering that they have a PC/PC/AT deficiency or perhaps worse a ‘genetic mutation’ (such as FVL/PGA); there are

also subsequent family issues when asymptomatic family members are also ‘discovered’ to have similar ‘genetic mutations’. Potentially worse is the risk that clinicians will over treat clinical conditions based on false positives for PC/PC/AT deficiency or the presence of FVL/PGA or aPL, e.g., by extending the duration of anticoagulant therapy for these ‘lifelong conditions’ and thus increased risk of eventual bleeding events [4, 15, 16]. Also important is that all the false diagnoses (e.g., PC/PC/AT deficiencies) will either remain in the patient’s clinical history files, or will need to be reversed by additional retesting. For example, once an individual is marked as being ‘PC/PC/AT deficient’, it becomes difficult to reverse this diagnosis, since the record of ‘deficiency’ is often retained forever within the patient’s clinical notes or within laboratory information systems.

Finally, in many cases, the utility of even a true-positive diagnosis remains limited. For example, these laboratory markers do not provide very high relative risk for thrombosis reoccurrence, so knowledge of their presence or absence provides only limited utility for advising on future risk. Moreover, discovery of true PC/PC deficiencies or FVL/PGA mutations do not in general alter clinical management, which in most cases involves short-term (3–6 months) anticoagulant therapy and avoidance of high thrombosis risk activities, which would be similar irrespective of the presence or absence of these markers.

In conclusion, thrombophilia testing is more likely to impact negatively on the health care of the people being tested, as well as the health care of their family members, than the positive impact that is promised by theoretical considerations [1, 2, 17], thereby leading the author to conclude on the general futility of thrombophilia testing.

Conflict of interest statement

Author’s conflict of interest disclosure: The author stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Received July 18, 2013; accepted July 19, 2013; previously published online August 12, 2013

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