Thrombin Generation Biomarkers Decline With Parenteral Anticoagulation—An Overlooked Means of Anticoagulation Monitoring?

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Abstract

Anticoagulation therapy is administered to patients to prevent or stop thrombin generation in vivo. Although plasma tests of in vivo thrombin generation have been available for more than 2 decades, they are not routinely used in clinical trials or practice to monitor anticoagulation therapy. We observed a fall in one such marker, the D-dimer antigen, in patients receiving anticoagulation therapy. We therefore conducted a systematic review of the medical literature to document the change in serum biomarkers of thrombin generation following the initiation of anticoagulation therapy. Using a defined search strategy, we screened PubMed and Embase citations and identified full-length articles published in English. Eighteen articles containing serial changes in 1 of 3 markers of thrombin generation (D-dimer antigen, thrombin–antithrombin complexes, and prothrombin fragment 1+2 antigen levels) in the 14 days following the initiation of anticoagulation were identified. Even though the assays used varied considerably, each of the 3 markers of thrombin generation declined in the initial period of anticoagulation therapy, with changes evident as early as 1 day after beginning therapy. These observations provide a rationale for further exploration of these markers as measures of the adequacy of anticoagulation using classic as well as novel anticoagulants. Particular patient groups that would benefit from additional means of monitoring anticoagulation therapy are discussed.

Keywords

coagulation monitoring, anticoagulation, D-dimer antigen, T-AT complexes, F1+2 antigen

Introduction

Anticoagulant drugs are used to prevent thrombosis or to treat patients with established thrombosis. Successful treatment halts the ongoing generation of thrombin, fibrin clot propagation, and generation of plasmin, thereby decreasing the risk of thromboembolism.

Heparin and vitamin K antagonists have been used to prevent and treat thrombotic diseases for decades. Because a given dose of these agents can have different effects upon laboratory tests of anticoagulation, health professionals have been taught that *anticoagulant monitoring* with laboratory tests (typically the prothrombin time and the activated partial thromboplastin time [aPTT]) is required when using these classic agents. The emphasis on maintaining test results "within the therapeutic range" has led many to assume that if the target blood clotting time is achieved, the patient's blood coagulation system has been adequately inhibited. Although this approach leads to successful prevention or treatment for most patients, it is not based on direct demonstration of anticoagulant efficacy in vivo. Each of these ex vivo laboratory tests has limitations,[1]

and in both clinical trials and practice, there are a small percentage of patients who experience progressive disease even when standard guidelines are followed.

Many newer anticoagulants have been developed that can be administered without monitoring. The lack of required routine laboratory monitoring simplifies the management for many patients and physicians. Coincident with the development of these drugs has been a drop-off in investigation of more sophisticated laboratory techniques for determining the efficacy of anticoagulant therapy. End points for determination of efficacy

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(or therapeutic resistance) are therefore confined to diagnostic imaging tests. Expert opinion, rather than clinical trial data, is the main source of guidance for the management of complex patients.[2]

A number of tests have been developed over the years to measure in vivo generation of thrombin. The first generation utilized functional assays to detect circulating fibrin monomers, using end points such as staphylococcal clumping,[3] protamine-induced paracoagulation,[4] ethanol-induced gelation,[5] or augmented plasmin generation by tissue plasminogen activator.[6] The second generation utilized radiolabeled fibrinogen and examined the effects of anticoagulation treatment upon the circulation time of fibrinogen in selected patients.[7] The third generation utilized immunoassays to quantify markers of coagulation system activation. Some target the products of thrombin cleavage of fibrinogen (ie, circulating fibrin monomers or fibrinopeptides),[8-10] while others measure peptides released when coagulation factor zymogens (such as Factors II, IX, or X) are converted to active enzymes.[11-13] Measuring thrombin–antithrombin (T-AT) complexes, which are formed following the in vivo generation of thrombin,[11] has also been informative regarding in vivo thrombin generation.

Another assay quantitates the cross-linked fibrin degradation product known as the D-dimer.[14] Unlike other assays, which measure products produced by thrombin alone, D-dimer generation depends upon the generation of both thrombin and plasmin. It is currently used to exclude venous thromboembolism (VTE), to diagnose and monitor disseminated intravascular coagulation, and to assist in determining the duration of anticoagulation for VTE.[15-18] Unlike all other tests that reflect in vivo thrombin generation, the D-dimer is widely available. It is not generally used, however, to monitor coagulation system activation in patients receiving anticoagulant drugs.

We observed a fall in D-dimer levels in complex hospitalized patients being treated with anticoagulation. Reasoning that laboratory tests of one or more thrombin generation biomarkers might be useful in certain patient subgroups, we conducted a search of the medical literature to determine whether others had systematically measured plasma levels of these biomarkers in patients starting parenteral anticoagulation. As described below, we identified a number of studies where serial measurements of 1 or more of 3 antigenic markers (prothrombin fragment 1+2 [F1+2], T-AT, and D-dimer antigen) were performed after the initiation of anticoagulation.

Methods

Systematic searches of the PubMed and Embase databases were performed by one of the investigators (C.P.M.) on August 26, 2016. All available articles from inception through the search date were included. The search strategy involved searching for combinations of keywords including "venous thromboembolism," "D-dimer," "T-AT," "F1+2," "anticoagulation," and numerous synonyms of these terms as well as related Mesh terms in PubMed and Emtree terms in Embase. The full search strategy is described in the

Supplemental Appendix. Reference lists of articles ultimately selected for inclusion were also hand-searched for eligible studies. Only peer-reviewed articles published in English were evaluated.

After screening titles and abstracts of studies identified by the database search, candidate studies identified by this initial screening were reviewed in full. Inclusion criteria were as follows: (1) the study population consisted of adult patients with newly diagnosed acute pulmonary embolism or deep vein thrombosis; (2) treatment of VTE in all patients was initiated using therapeutic dosing of anticoagulant drugs (including heparin products with or without the simultaneous initiation of warfarin, direct oral anticoagulants, parenteral direct thrombin inhibitors, and fondaparinux); (3) D-dimer, T-AT, and/or F1+2 measurements were taken within 1 day before, or at the same time as, the initiation of anticoagulation and at least once more within 14 days following initiation of anticoagulation. Studies in which patients were already receiving anticoagulation therapy at the time of initial measurement of D-dimer, T-AT, or F1+2 were excluded, as were studies utilizing prophylactic dose anticoagulation regimens. The 14-day maximum duration prior to the second laboratory draw was used because of our interest in defining changes in markers of thrombin generation during the acute treatment period of VTE. Both prospective observational studies and randomized controlled trials were eligible for inclusion. There was no requirement that levels of D-dimer, T-AT, or F1+2 were the primary outcome measure of the study. One study of potential relevance was not included because a full-text version could not be obtained, even after attempting to contact the author of the study.

Data were extracted from studies by one of the investigators. A standardized form was used to extract first author name, publication date, method of deep vein thrombosis or pulmonary embolism diagnosis, anticoagulation treatment used, population size, and comparator groups, if any. An additional form was used to abstract values of D-dimer, T-AT, and F1+2 at various time points relating to the start of anticoagulation. For several studies in which data were presented graphically in plots but not numerically, estimated numerical values were extracted from figures using the program WebPlotDigitizer version 3.9.[19] Data presented as means were used to construct plots showing the trends in changes of these parameters over time during anticoagulation therapy; data presented as medians were presented individually.

Results

The PubMed literature search identified 2247 candidate papers, and the Embase search identified 2557 candidate papers. Duplicates from these 2 searches were identified and discarded, leaving 4059 unique articles. Screening and study selection as described above resulted in the identification of 20 articles[20-39] meeting the inclusion criteria (Figure 1). Two articles[29,32] reported data found in other included reports[31,34] and were not included in the final analysis. Of the 18 included studies that
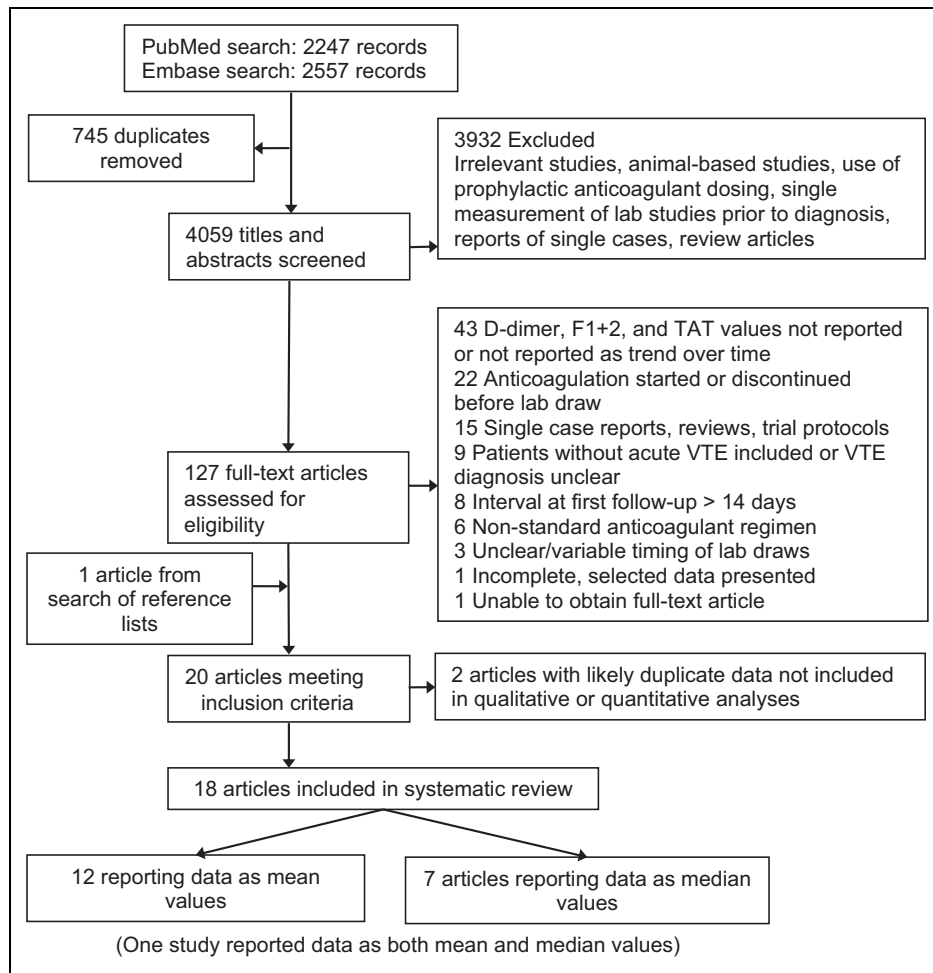
Figure 1. Flow diagram of systematic review. Figure adapted from PRISMA.[66] F1+2 indicates prothrombin fragment F1+2; T-AT, thrombin–antithrombin complex; VTE, venous thromboembolism.

tracked serial changes in one or more markers of coagulation system activation after the initiation of treatment for VTE, 16 reported serial D-dimer levels, 12 reported serial T-AT levels, and 10 reported serial F1+2 antigen levels (Table 1). The studies differed in their sampling frequency, drug utilization, duration, specific immunoassays utilized, and reporting of outcomes. Some of the studies reported their findings in terms of the mean levels, while others reported median levels. After extracting data from all papers, we found that the range of values reported in each study varied considerably from the others. We therefore normalized the data by dividing each value by the initial (day 0) level.

Of the included 18 studies, 12 reported the mean values of laboratory markers for the participants studied and 7 reported median values. (One study reported its D-dimer results as both mean and median values; herein we include the mean D-dimer results only.) The mean values for all participants studied in each of the 12 studies were averaged and normalized and are shown in Figure 2. Data for different treatment groups in 1 study[35] could not be averaged as exact numbers of participants were not provided by the study; results in the 3 treatment

groups were similar, and data from only 1 of the groups are presented. The solid line in each panel shows the best fit of the aggregated data by linear regression analysis. Levels of each of the 3 biomarkers showed a decline upon the initiation of anticoagulation.

The data of the 7 studies that reported median results were normalized, as shown in Figure 3. Some of the studies reported data for subsets of the study population only and did not report median values of the entire study population; we therefore show the data as reported in the paper. Patients were often grouped based on treatments given (typically low-molecular-weight heparin [LMWH] vs unfractionated heparin) or based on response to treatment. "Responder" in these studies typically indicates stabilization or shrinkage in measured thrombus or the absence of VTE recurrence, though definitions, terminology, and methods of measurement varied by study. Although there were a few exceptions, most markers of thrombin generation in most patient groups declined in these studies during the course of anticoagulation treatment, as was observed in the studies that reported data as mean values.

Table 1. Studies With Serial Measurements of D-dimer, FI+2, and/or T-AT Complexes During Treatment of Acute VTE With UFH or LMWH Listed in Chronological Order.

Study	VTE Diagnosis and Method	Treatments and Sample Size	Patient Groups With Thrombin Formation Surrogate Data Reported	Thrombin Formation Surrogates Measured (With Statistic Reported)
Harenberg et al[20]	DVT by duplex ultrasound	IV UFH adjusted to goal anti-Xa level(n = 26), subcutaneous unspecified LMWH type adjusted to goal anti-Xa level (n = 24)	UFH (n = 26), LMWH (n = 24)	D-dimer, T-AT (mean)
Speiser et al[21]	DVT by phlebography	IV UFH followed by transition to subcutaneous UFH, both adjusted to goal thrombin clotting time (N = 13)	All patients (N = 13)	D-dimer, T-AT (mean)
Estivals et al[22]	DVT by contrast phlebography	IV UFH with dose adjusted to goal aPTT (n = 13), subcutaneous UFH (n = 1), dalteparin (n = 1)	All patients (N = 15)	D-dimer, FI+2, T-AT (mean)
Amelsberg et al[23]	DVT by phlebography	IV UFH adjusted to goal aPTT (N = 14)	All patients (N=14)	D-dimer, FI+2, T-AT (median)
DVTENOX[24]	DVT by venography	IV UFH adjusted to goal aPTT (n = 44), enoxaparin (n = 47)	All patients (N = 91), UFH (n = 44), enoxaparin (n = 47), "worsened" DVT (n = 9), "improved" DVT (n = 44), "stationary" DVT (n = 38)	D-dimer, FI+2, T-AT (mean)
Mesters et al[25]	DVT by duplex ultrasound	IV UFH adjusted to goal aPTT (N = 50)	No progression (n = 44), progression (n = 6)	D-Dimer, FI+2, T-AT (median)
Arcelus et al[26]	DVT by duplex ultrasound	IV UFH adjusted to goal aPTT (N = 35) (12 patients already on treatment, unspecified duration, prior to laboratory draws)	All patients (N = 35), complete resolution (n = 20), incomplete resolution (n = 15), proximal DVT (n = 29), distal DVT (n = 6)	D-Dimer (mean)
Minnema et al[27]	PE by lung scan, angiography, and duplex ultrasound	IV UFH adjusted to goal aPTT (N = 28)	All patients (N = 28)	D-dimer, FI+2, T-AT (median for all, mean also for D-dimer)
Bounameaux et al[28]	DVT by venography	IV UFH adjusted to goal aPTT (n = 44)	All patients (n = 44)[a]	FI+2, T-AT[c] (median)
Meissner et al[29]	DVT by duplex ultrasound	IV UFH or LMWH (n = 42), warfarin only (n = 2), no treatment (n = 13), unknown (n = 4)	All patients (N = 61 initially with some dropout), no recurrence (n = 35), recurrence (n = 26)	D-dimer, FI+2 (median)
Peternel et al[30]	DVT by duplex ultrasound	Subcutaneous UFH adjusted to goal aPTT (n = 28), dalteparin (n = 31)	UFH (n = 28), dalteparin (n = 31)	D-dimer, FI+2, T-AT (median)
Harenberg et al[33]	DVT by ascending venography	IV UFH adjusted to goal aPTT (n = 41), certoparin (n = 48)	Certoparin improved (n = 42), UFH improved (n = 29), certoparin worsened (n = 6), and UFH worsened (n = 12)	D-dimer (mean)
Kakkar et al[32]	DVT by phlebography	IV UFH adjusted to goal aPTT (n = 375), reviparin BID (n = 388), reviparin daily (n = 374)	UFH (n = 375), reviparin BID (n = 388), and reviparin daily (n = 374)	D-dimer, FI+2, T-AT (median)
Meissner et al[31]	DVT by duplex ultrasound	IV UFH or LMWH (n = 53), warfarin only (n = 5), no treatment (n = 8), unknown (n = 5)	All patients (N = 71 initially with some dropout)	D-dimer, FI+2 (mean)
Breddin et al[34]	DVT by phlebography	IV UFH adjusted to goal aPTT (n = 375), repivarin BID (n = 388), repivarin daily (n = 374)	Responder (n = 466) and nonresponder (n = 419)	D-dimer, FI+2, T-AT (median)
Hoppensteadt et al[35]	DVT, diagnostic method not specified	IV UFH adjusted to goal aPTT (n = 5-10), tinzaparin for 7 days (n = 5-10), tinzaparin for 90 days (n = 5-10)	UFH (n = 5-10), tinzaparin for 7 days (n = 5-10), tinzaparin for 90=days (n = 5-10)	T-AT (mean)
Schutgens et al[36]	PE by VQ scan	UFH adjusted to goal aPTT (n = 19), dalteparin (n = 18)	UFH (n = 19) and dalteparin (n = 18)	D-dimer, FI+2, T-AT (median)
Chen et al[37]	PE by VQ scan or CT angiography	UFH adjusted to goal aPTT (n = 57) or nadroparin (n = 57)	UFH (n = 57) or nadroparin (n = 57)	D-dimer (mean)
Ozlu et al[39]	PE by CT angiography	UFH adjusted to goal aPTT (n = 16)	All patients (n = 16)[b]	D-dimer (mean)
Sartori et al[38]	DVT by ultrasound	Enoxaparin (N = 59)	All patients (N = 59), postthrombotic syndrome (n = 12), no postthrombotic syndrome (n = 46)	D-dimer (mean)

Abbreviations: aPTT, activated partial thromboplastin time; BID, twice daily; CT, computed tomography; DVT, deep vein thrombosis; FI+2, prothrombin fragment FI+2; IV, intravenous; LMWH, low-molecular-weight heparin; PE, pulmonary embolism; T-AT, thrombin–antithrombin complex; UFH, unfractionated heparin; VTE, venous thromboembolism; VQ scan, ventilation-perfusion scintigraphy.

[a]Napsagatran-treated groups not included.

[b]Group treated with delayed warfarin dosing not included.

[c]T-AT data could not be accurately abstracted from the figures in this source and was thus not included.

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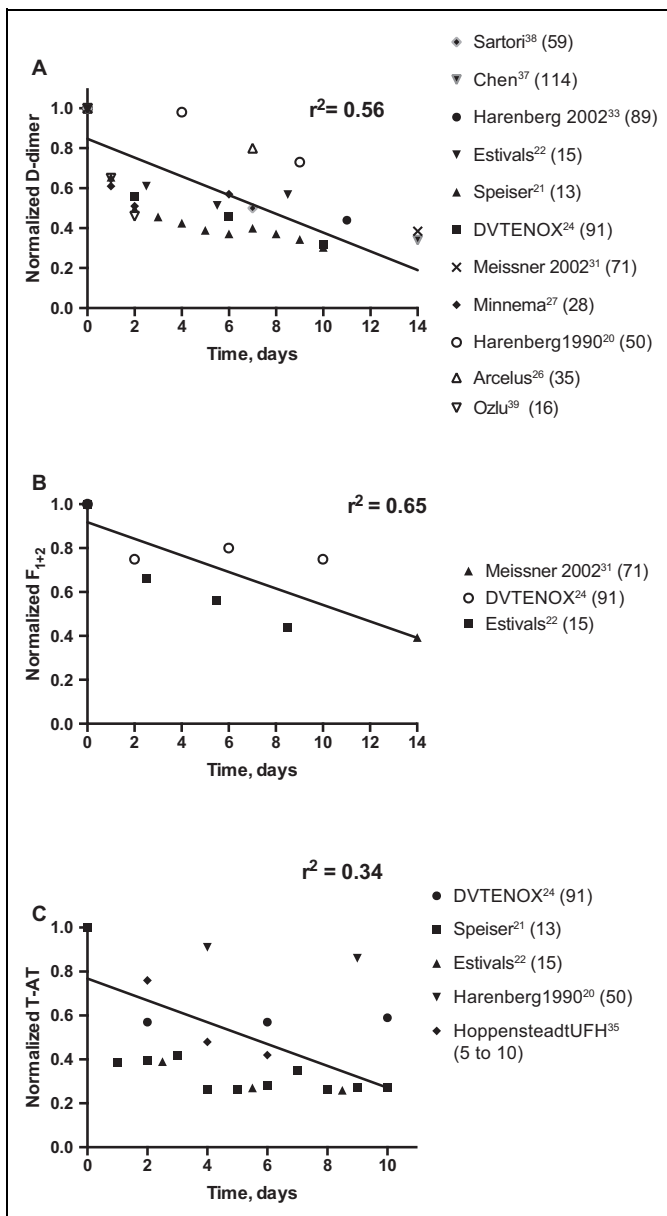
Figure 2. Serial measurements of markers of coagulation system activation in patients receiving UFH or LMWH for venous thromboembolism. A, Normalized D-dimer levels following initiation of anticoagulation in studies reporting mean values. The number of participants in each study is indicated in parentheses. The solid line indicates the best fit to the aggregate data obtained by linear regression analysis. B, Normalized F1+2 levels following initiation of anticoagulation in studies reporting mean values. The number of participants in each study is indicated in parentheses. The solid line indicates the best fit to the aggregate data obtained by linear regression analysis. C, Normalized T-AT complex levels following initiation of anticoagulation in studies reporting mean values. The number of participants in each study is indicated in parentheses. The solid line indicates the best fit to the aggregate data obtained by linear regression analysis. Figure has been adapted by authors from figures in sources indicated. F1+2 indicates prothrombin fragment F1+2; LMWH, low-molecular-weight heparin; T-AT, thrombin–antithrombin complex; UFH, unfractionated heparin.

Discussion

In our practice, we observed a serial fall in D-dimer antigen levels in patients with VTE treated with anticoagulation. We found this intriguing, for it suggested that sequential D-dimer measurements might provide a means of confirming that a given dose of anticoagulant is effective at inhibiting coagulation system activation in an individual patient. Although such confirmation is not needed for the majority of patients who are treated with standard anticoagulation protocols, there are subgroups of patients where optimal management strategies are not well established. In order to determine whether our observations were unique to our practice setting (and current laboratory methodology), we performed a systematic review of the literature. We found that 3 laboratory markers of coagulation system activation (D-dimer, T-AT, and F1+2) were measured serially in several studies. We extracted data from the papers to allow a graphical analysis of the changes in the levels of these markers during the initial phase of anticoagulation. A majority of the papers reported data as mean values, which allowed us to combine their results to obtain correlation coefficients for changes in biomarker levels as a function of time during short-term anticoagulation. Although the results from each study are not identical, the overall agreement is quite striking, considering that there was no coordination in the planning and/or performance of these studies. Patient subtypes, coexisting conditions, methods of drawing blood samples, kits used to measure thrombin generation markers, and presence or absence of inflammatory markers are but some of the many factors that could be invoked why the results were not identical.

The D-dimer antigen level was measured more often than the F1+2 or T-AT antigen levels. An excellent detailed review of the biochemistry of D-dimer formation and the tests developed for its quantitation has been published.[15] In brief, the antigen detected in the D-dimer assay is formed from the sequential actions of thrombin, factor XIIIa, and plasmin upon fibrinogen. Importantly, coagulation of plasma in vitro does not result in an increase in D-dimer antigen concentration,[40] suggesting that poor venipuncture technique or drawing of blood through indwelling venous catheters may be less likely to cause artifactual elevation in the D-dimer level. (Studies are needed to confirm this conjecture, however, particularly in regard to catheters in place for a number of days.) Unlike the other tests, the D-dimer is widely available and its expanded use for monitoring anticoagulation would not pose a hardship for most laboratories.

The decline in all 3 markers over the first 14 days of anticoagulation suggests that one or more might be used as biomarkers for the effectiveness of anticoagulation. Since F1+2 is metabolized by the kidney, fluctuations in renal function may render it a less useful test in medically complex patients. Both the F1+2 and T-AT levels are very sensitive to activation of blood coagulation, and it is
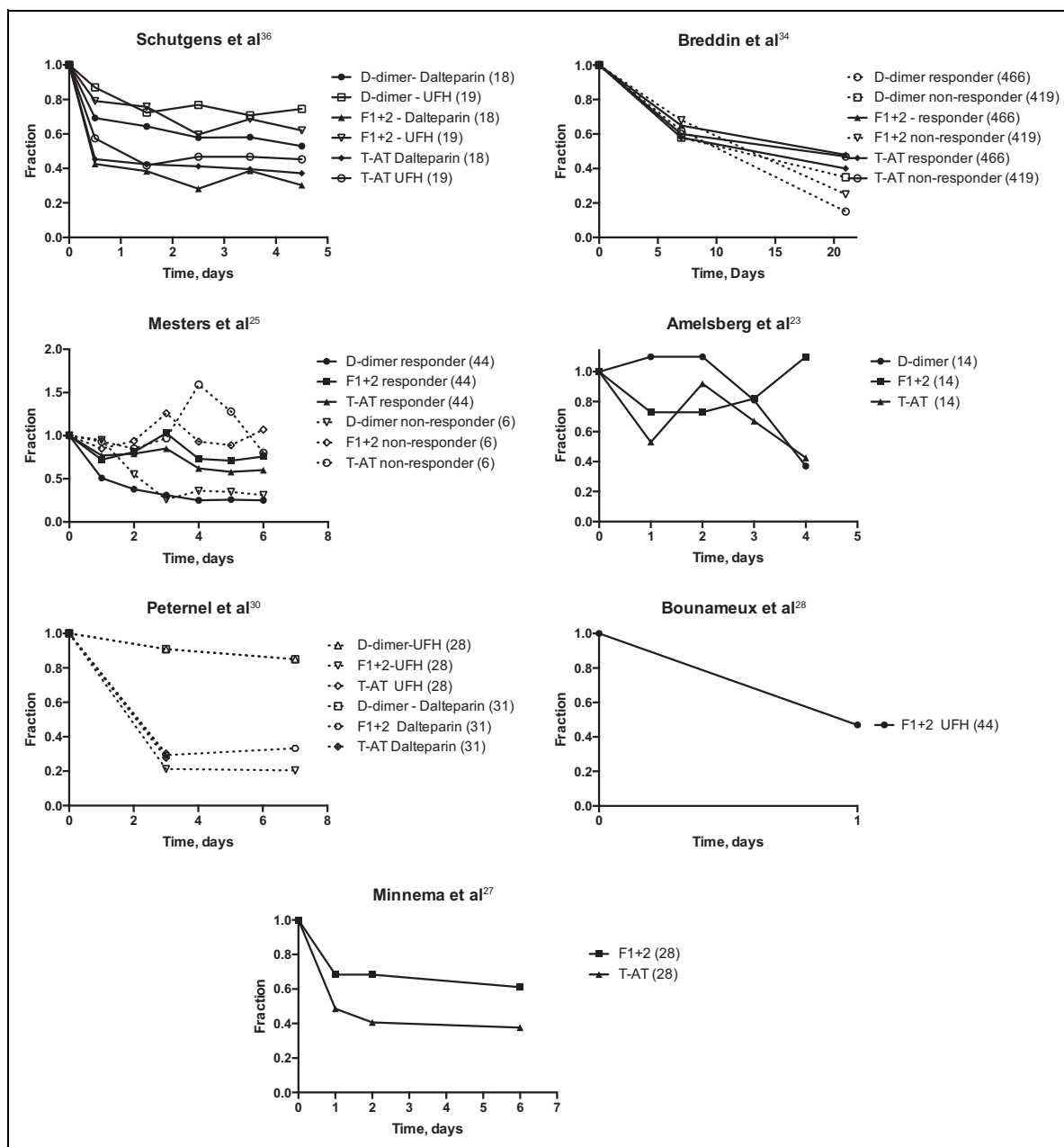
Figure 3. Serial measurements of markers of coagulation system activation in studies reporting median values for patients receiving anticoagulation for VTE. Number of patients in each treatment group are in parentheses. Figure has been adapted by authors from figures in sources indicated. F1+2 indicates prothrombin fragment F1+2; T-AT, thrombin–antithrombin complex; UFH, unfractionated heparin; VTE, venous thromboembolism.

possible that routine hospital phlebotomy (rather than the careful techniques used in the published studies) may result in spuriously elevated results, particularly in those with poor venous access or who require blood draws through indwelling intravenous catheters.

Using biomarkers to monitor anticoagulation efficacy will not be needed in all patients but may be useful in specific subgroups of patients. We discuss briefly 5 groups of patients whose care might be improved by the use of thrombin generation biomarkers to optimize anticoagulation therapy.

Atrial Fibrillation

A meta-analysis[41] of published trials comparing the new oral anticoagulant agents (NOACs) with warfarin in patients with atrial fibrillation showed that the pooled risk of stroke or embolic events was 3% in patients treated with NOACs and 3.8% in patients treated with warfarin. It has long been recognized that atrial fibrillation is associated with an increase in markers of coagulation system activation and that persistent elevation may be associated with therapeutic failure.[42,43] Adjustment of drug therapy to optimize a marker of in vivo

anticoagulation, such as the D-dimer, may reduce the risk of stroke in this patient population, or high-risk subgroups.

Left Ventricular Assist Devices

Left ventricular assist devices are an increasingly common intervention utilized for patients with heart failure. Although the Edmonton Anticoagulation and Platelet Inhibition Protocol, which is based upon thromboelastography, is used for pediatric monitoring,[44] there is little (if any) consensus on the management of adults.[45] There is considerable discordance between anti-Xa levels and aPTT levels in these patients,[46] leaving open the question (as with general treatment of VTE) of which laboratory "target" should be used. Personal observations suggest that in some patients, pump thrombosis may be preceded by a rise in D-dimer levels. Use of thrombin generation biomarkers may allow anticoagulant medications to be administered by laboratory testing that reflects the variable coagulant–anticoagulant balance in these complex patients.

Obstetric Patients

Although it has been recognized for a number of years that the dose of LMWH required by pregnant patients to maintain "therapeutic" plasma levels increases during pregnancy, there are no data to indicate that maintaining such levels improves outcomes. D-dimer levels rise during pregnancy,[47-49] and several studies have examined D-dimer levels in pregnant women receiving either prophylactic or therapeutic doses of LMWH. Although the sample sizes have been small, the studies fail to show dramatic decreases in D-dimer levels in treated patients.[50-52] As the studies did not uniformly increase LMWH dosing during pregnancy, no firm conclusions can be drawn from these observations. Trials of individualized heparin dosing using measures of coagulation system activation (that take into account physiologic changes that occur in healthy women) may provide an avenue for future investigation.

Antiphospholipid Antibody Syndrome

Some patients with antiphospholipid antibody syndrome experience recurrent thrombosis despite seemingly adequate anticoagulation treatment. There is little data to indicate how they are best managed.[53] The only studies that attempt to provide guidance to managing clinicians were based upon the use of higher international normalized ratio target ranges,[54,55] and uncertainty remains about the benefits of this approach for the majority of patients.[56,57] A recent report of 151 lupus anticoagulant-positive patients indicates that 20% had recurrent events, half of which were arterial.[58] A variety of "antithrombotic" and "anticoagulant" therapies are used in these patients. Monitoring the degree of ongoing thrombin generation in such cases might provide insights into the roles of both anticoagulant and antiplatelet drugs in fibrin generation.

Patients With Cancer

The management of patients with cancer having recurrent thrombosis remains a difficult problem,[59] with few data to drive decision-making. Changing treatment regimens, an evolving inflammatory response to tumor burden, and coexisting clinical morbidities (such as infectious episodes) characterize their course. An individualized (or "personalized") approach to care, utilizing coagulation system activation markers to decide upon doses of anticoagulant drugs, may result in fewer recurrences in those who experience thrombosis while receiving standard doses of anticoagulants.

Our systematic literature review includes patients with acute VTE for whom only unfractionated heparin or LMWH was used for initial treatment. We anticipate that a decrease in markers of coagulation system activation would also be found if large numbers of patients are studied prior to and after the initiation of therapy using other types of anticoagulants, such as parenteral or oral direct thrombin and Xa inhibitors. Indeed, reports documenting a decline in D-dimer in small numbers of patients treated with a number of the novel anticoagulant drugs have appeared, including argatroban,[60] lepirudin,[61] dabigatran,[62] rivaroxaban,[63] apixaban,[64] and edoxaban.[65] With greater awareness of the clinical relevance of such measurements, monitoring D-dimer levels may provide a new standard for assessing anticoagulation efficacy for new drugs or for patients who are outside the norms of those studied in drug registration trials. Routine monitoring of the majority of patients, who do well under current guidelines, is unlikely to be cost-effective.

Although large controlled studies of clinical outcomes are always desired before adopting new clinical management tools, the financial and logistical requirements of performing studies of even a single thrombin generation biomarker with the ever-increasing number of anticoagulant drugs on the market would be formidable. Establishing that one or more biomarkers of coagulation system activation provide a suitable surrogate for relevant clinical end points would allow investigators to conduct studies in patient subsets during the routine administration of medical care, without pharmaceutical industry backing. Without such studies, clinicians will continue to be dependent upon expert opinion and observations from large drug company-sponsored registration studies that (necessarily) do not include smaller patient populations of interest. Using each patient's baseline values may be a way of overcoming the objection that tests for thrombin generation biomarkers are not standardized. Although following markers of coagulation system activation will not address the problem of bleeding in patients treated with these drugs, many specialties of medicine may find it helpful to utilize physiologically based measures of anticoagulation efficacy.

Declaration of Conflicting Interests

Funding

Supplemental Material

Supplementary material for this article is available online.

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