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HAEMATOLOGY

Coagulation studies: achieving the right mix in a large laboratory network

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Summary

Basic coagulation tests, activated partial thromboplastin time (APTT), prothrombin time (PT) and the related international normalised ratio (INR), are performed frequently in hospital settings. From a laboratory perspective, unexpected abnormal results require further action; either informing the ordering clinician, or second line testing to determine the underlying cause. To streamline laboratory workflow, a new system of expert laboratory rules was implemented. The medical implications of this new laboratory system are evaluated here.

The electronic ordering system was updated to mandate clinical information regarding the presence of an anticoagulant, or 'no anticoagulant'. When the PT or APTT were abnormal, and no anticoagulant was reported, second line testing was automatically performed. The second line tests performed were: mixing studies, fibrinogen and thrombin time. Any sample with a mixing study that did not completely correct, or fibrinogen <1.0 g/L, or INR >7.0, was flagged for clinical review by the laboratory haematology registrar.

In a 17-month period there were 362,692 APTT, PT/INR and fibrinogen tests performed. Of these, 14,160 (3.9%) were abnormal with either no reported anticoagulant, or an unknown anticoagulant status. A total of 934 (0.3%) were referred for review by the haematology registrar. Three (<0.001%) cases received altered medical management as a result of the haematology registrar review. In hospital settings, most abnormal coagulation studies are anticipated by the ordering clinician. Unexpected abnormal coagulation results of clinical significance are rare. Automated second line coagulation testing and medical review improves laboratory workflow without compromising patient safety.

Key words: Coagulation; mixing studies; laboratory information system; reflex testing; expert rules.

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INTRODUCTION

Effective communication between clinicians and the laboratory forms an important part of patient care. This includes both the provision of appropriate clinical information by clinicians to the laboratory, as well as prompt notification of abnormal results by the laboratory to the ordering clinician.[1] There is obviously a delicate balance required for the latter scenario; many blood test results, especially in the acute hospital setting, are expected to be abnormal, and a high frequency of phone calls from the laboratory to the requesting clinician provides little benefit to the clinician, laboratory staff, or the patient.

At our institution, basic coagulation studies—activated partial thromboplastin time (APTT) and prothrombin time (PT)—are performed frequently. A significant proportion of these tests are identified as 'abnormal', although often due to the presence of an anticoagulant given therapeutically. Prior to 2017, any abnormal PT or APTT without accompanying clinical information alerting to the potential presence of an anticoagulant, resulted in verbal notification of abnormal results to the requesting clinician. This practice resulted in a significant number of verbal notifications due to initial poor communication from the clinician to the laboratory.

Attempts to improve this communication by including a mandatory response field querying the presence of an anticoagulant in the electronic coagulation study request, ultimately provided limited success because paper forms were still in use, and so could bypass the mandatory eOrder (electronic order) requirement. There were also concerns that ongoing increasing usage of direct oral anticoagulants (DOACs) would increase the likelihood of near-misses of truly significantly abnormal coagulation results. In our laboratory dabigatran and rivaroxaban both result in prolongation of APTT and PT/APTT, respectively; these results do not correct when mixing studies are performed, and thus appear as 'inhibitors'. Such results, in the absence of knowledge around anticoagulant therapy, would routinely require communication to the clinician.[2,3] On the other hand, true (factor) inhibitor samples, yielding similar laboratory test results, might be missed, given wide usage of such

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anticoagulants. Therefore, a new system of expert rules for coagulation test result verification and reporting was implemented.

This report focuses on an analysis of some outcomes arising from the implementation of this new rules system, and namely those 'abnormal' test results referred for clinical haematology review.

METHODS

Coagulation testing laboratory rule set

The new system of rules as established and implemented is reported elsewhere.[4] In brief, the system was dependent on provision of accurate anticoagulation history, which was facilitated by a mandatory field for clinicians to enter when placing an eOrder. Any prolonged PT or APTT that was not explained by the pre-disclosed history of anticoagulant use was then subject to automated, or 'reflex', second-line tests including an immediate mixing study (i.e., without incubation), fibrinogen and thrombin time. If the mixing studies corrected, an automated electronic report was released along with the results informing the clinician of the possible relevance of the results [e.g., potential factor(s) deficiency]. If the mixing studies failed to correct, or the fibrinogen was significantly reduced, the results were released to the electronic medical record and referred for a review by the haematology registrar to provide additional advice to the requesting clinician as indicated.

Rationale for the rule set

PT and APTT

Basic coagulation studies, APTT and PT, serve as the main initial investigations of haemostasis. Both the PT and APTT assess the ability of the patient's plasma to form a fibrin clot *in vitro* when exposed to specific activators under certain conditions. Arguably, one main purpose of these tests is to monitor the effect of anticoagulants. The PT, and the related international normalised ratio (INR), assess the presence of vitamin K antagonists (VKA) such as warfarin.[5] The APTT assesses unfractionated heparin, and other direct anticoagulants including bivalirudin and argatroban.[6–8] However, particularly within in-patient settings, these tests are frequently ordered for purposes other than anticoagulation monitoring.[9,10] A significant proportion of in-patient testing of PT and APTT are performed without clearly documented indication, or as a 'routine' prior to invasive procedures.[10] However, testing PT and APTT prior to a range of invasive procedures does not predict bleeding risk.[11]

Accepted indications for testing PT and APTT are: to assess the presence and/or effect of a known or suspected anticoagulant, as a marker of synthetic liver disease, in severe sepsis or disseminated intravascular coagulation (DIC), in the assessment of a patient who is actively bleeding, or in a patient with a suspected bleeding disorder.[9] It must be emphasised that the PT and APTT are only initial screening tests in the investigation of haemostasis. Therefore, the new laboratory testing rules automatically initiate performance of second-line tests if the clinical information provided does not explain the abnormal APTT or PT.

Mixing studies, thrombin time and fibrinogen

The presence of prolonged PT and/or APTT, without a clear clinical cause, should be further investigated with a mixing study.[12,13] Useful additional second-line investigations are fibrinogen and thrombin time.[13,14]

Mixing studies are performed by mixing the patient (test) plasma in a 50:50 ratio with plasma known to have a normal concentration of coagulation factors. If a patient is deficient in a coagulation factor, the prolonged APTT and/or PT will 'correct'. This correction should still occur, even in the presence of a factor level of zero in the patient plasma. Conversely, if there is an inhibitor (auto or alloantibodies) or anticoagulant present in the patient's plasma, the activity of the inhibitor/anticoagulant will prevent the coagulation factors from functioning normally. Therefore, the presence of an inhibitor results in mixing studies that do not fully correct. It is important to note that immediate mixing studies (i.e., without incubation) will not detect all factor VIII inhibitors. To reliably detect factor VIII inhibitors, mixing studies need to be tested after incubation at 37°C for 2 h.[12]

There are several different methods of interpreting mixing studies. Examples include the Rosner index, 'correction' only if complete correction into the normal reference range, consideration of the per cent correction, as well as other calculations.[13] The goal of each method of interpretation remains to differentiate between the presence of an inhibitor or factor deficiency. There is no clear evidence demonstrating that one method is superior. Ultimately mixing studies are not definitive investigations, but they indicate the direction in which further investigations should occur to identify the cause of abnormal PT and/or APTT.[10] Our laboratory defines 'correction' as normalisation of the PT and APTT into the reference range. This interpretation was selected in part because it is supported by the latest of the lupus anticoagulant (LA) guidelines, and in part because it could be easily applied in the rules set.[15] An internal (unpublished) study confirmed that this approach provided similar outcomes to more complex approaches.

Thrombin time is a useful second-line investigation of prolonged PT and APTT.[14] It is significantly prolonged in the presence of heparin, bivalirudin, argatroban and dabigatran. It is prolonged to a lesser degree in hypofibrinogenaemia, DIC, liver disease, significant hypoalbuminaemia and high levels of paraproteins.

Fibrinogen is another useful second-line investigation of prolonged PT and APTT.[14] It is reduced in DIC, synthetic liver dysfunction, in inherited disorders of fibrinogen production and due to thrombolytic agents and other drugs.

Haematology registrar review

Most basic coagulation studies in our laboratory are performed on in-patients, via an electronic ordering system. The electronic ordering system was updated to mandate the ordering clinician provide information on history of anticoagulant use by selecting a choice from a list of anticoagulants, or choosing 'no anticoagulant'. Within 12 months of implementing the updated electronic ordering system, the laboratory transitioned to reflex performance of mixing studies, fibrinogen and thrombin time. Any mixing study not completely correcting to within the laboratory's normal range for PT and/or APTT was flagged, and a haematology registrar review was required. This review was additional to the Critical Alert values and protocol already in place at our facilities. The Critical Alert values included fibrinogen <1.0 g/L or INR >4.5, which is in line with international guidance and which requires the laboratory to contact the requesting clinician.[16] In addition, any fibrinogen <1.0 g/L or INR >7.0 was also flagged for haematology registrar review. The value of 1.0 g/L for fibrinogen was chosen as this represents a standard cut-off value for 'critical review' by a number of guidelines, and values below this are associated with increased bleeding risk.[16] The value of INR >7.0 for registrar review was chosen as an intermediate threshold (between our critical value of 4.5 and the local national guideline upper threshold level of INR=10 for reversal of warfarin in a non-bleeding patient).[17] The intention here was to alert haematology registrars to review patient status independent of any pre-known clinical situation, and as an additional safety step to our critical result policy (INR=4.5), also being conservatively risk averse in comparison to an upper threshold of 10 in guidelines by Tran *et al.*[17]

During the first 5 months of system implementation, from 25 July 2016 onwards, results with INR >7.0 or fibrinogen <1.0 g/L were flagged for registrar review ('Stage 1'). Thereafter, any abnormal result with mixing studies that failed to correct was referred for registrar review ('Stage 2'). At this point, the laboratory staff were no longer required to verbally notify the clinician of these abnormal results.

This audit examines the results that required review by the haematology registrars from 25 July 2016 to 29 December 2017.

RESULTS

Figure 1 summarises the results during Stage 1 which include a total of 105,162 PT/INR and 4403 fibrinogen tests, of which 2.9% (3213) were identified as abnormal results not due to reported anticoagulants. Of the 3213, 3.6% (115) were flagged for review by haematology registrar, 45 (39%) of which were due to fibrinogen <1.0 g/L. The causes of hypofibrinogenaemia were apparent in all cases, including liver dysfunction, critical bleeding and associated massive

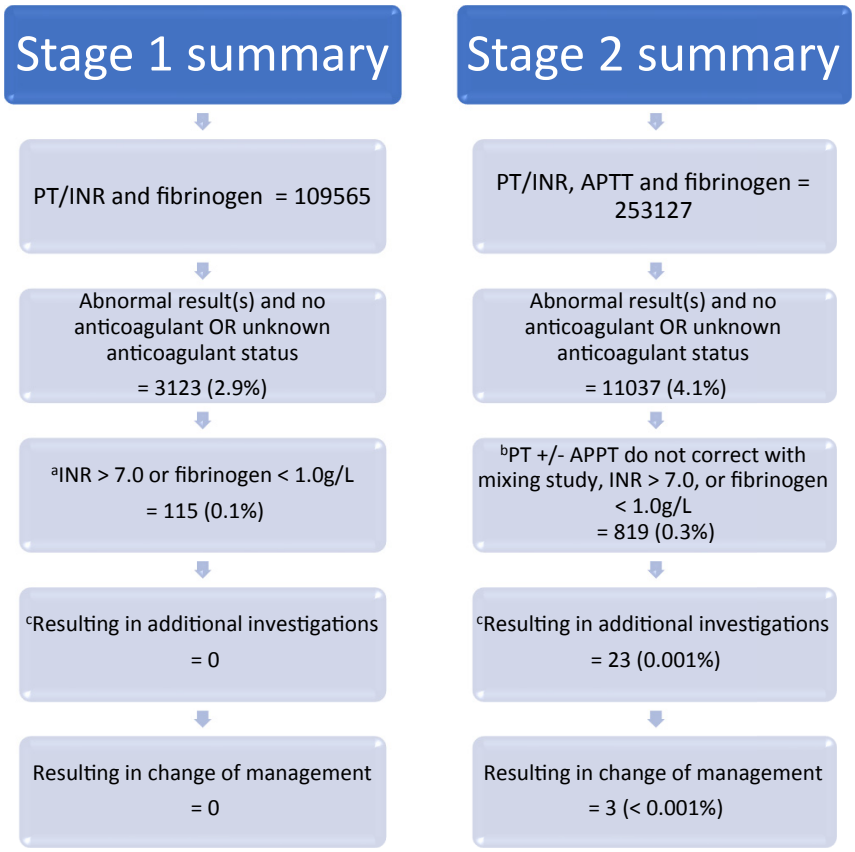

Fig. 1 Summary of results from Stage 1 and Stage 2 of the rule-set implementation. [a]Stage 1: Haematology registrar review if INR >7.0 or fibrinogen <1.0 g/L. [b]Stage 2: Haematology registrar review if INR >7.0 or fibrinogen <1.0 g/L or APTT ± PT fail to completely correct on immediate mixing study. [c]Additional investigations deemed to be solely due to the presence of the haematology registrar review.

transfusion, extreme prematurity, thrombolysis, plasma exchange with albumin, haemophagocytic lymphocytic histiocytosis, and L-asparaginase (used in the treatment of acute lymphoblastic leukaemia). The remaining 70 cases were due to INR >7.0. They were predominately due to isolated supratherapeutic warfarin. Some cases were due to supratherapeutic warfarin with intercurrent illness or DIC. Four (3.5%) of the abnormal results were due to pre-analytic error. Cases with pre-analytic error were categorised as a result of repeat testing on a fresh sample that was normal, without any intervening intervention.

In the first 5 months, there was no evidence that the provision of a haematology registrar review altered further investigation or led to alternative patient management. This is to some degree confounded by the fact that these reviews only occurred within standard working hours (8 am–5 pm Monday to Friday). Outside of these hours, the on-call haematology registrar may have been called for further advice on the interpretation of the results and further management, but these interventions were not recorded.

Stage 2 of the rule-set implementation followed; Fig 1 summarises this 12-month period from January to 29 December 2017, during which time there were 253,127 APTT/PT/INR tests and 13,390 fibrinogen levels performed. In total, 11,037 (4.1%) abnormal results were filtered by the algorithm. These resulted in 819 (7.4%) abnormal results requiring haematology registrar review.

Of the 819 cases of abnormal coagulation results, 789 (96.3%) had an easily identifiable cause. The causes were assessed by review of previous results or coagulation orders, or by review of the patient's clinical history available on the electronic medical record. A haematology registrar review would not have been required if the correct clinical information had been initially provided upon test request in 152 cases (19%).

Twenty-three cases (3%) had additional investigations performed as a result of the haematology registrar review. These additional investigations included: factor levels, D-dimer, lupus anticoagulant, von Willebrand factor testing and anticoagulant drug level testing. However, management was changed in only three (0.35%) of these patients. The APTT reagent used in our laboratories, in line with international recommendations, is relatively lupus insensitive, and therefore the occurrence of non-specific lupus inhibitors leading to isolated prolonged APTT was not observed in this cohort.[15] One patient demonstrated a prolonged APTT that corrected to within 1 second of the normal range, and proceeded to expedited factor level testing, confirming factor 12 deficiency. This allowed the patient to progress to intended surgery that was otherwise at risk of being deferred. One patient was taking rivaroxaban and had an acute kidney injury and was recommended to convert to warfarin. One patient had sepsis and liver failure and fresh frozen plasma was administered after discussion with the treating clinical team. In a small proportion of events (3.5%), there was no access to the patient's medical record and the clinical information was insufficient to determine the cause of the abnormal coagulation studies.

Clinical reasons identified that lead to mixing studies which failed to completely correct, or where INR was >7.0 or fibrinogen <1.0 g/L, are summarised in Table 1.

Table 1 Summary of outcomes for all cases (n=934) reviewed by haematology registrar

Cause[a]	Total coagulation studies (%)
Warfarin supratherapeutic	448 (48%)
Heparin infusion not reported	109 (11.7%)
Dabigatran not reported	47 (5%)
Rivaroxaban not reported	25 (2.5%)
Apixaban not reported	2 (0.2%)
Argatroban/bivalirubin not reported	3 (0.3%)
Hepatic impairment	93 (10%)
Pre-analytic error	36 (3.9%)
Trauma or massive transfusion	34 (3.6%)
Disseminated intravascular coagulation	27 (2.9%)
Snake bite	4 (0.4%)
Multiorgan failure	4 (0.4%)
Plasma exchange	7 (0.7%)
L-asparaginase	14 (1.5%)
Extreme prematurity	13 (1.4%)
Sepsis, bone marrow transplant patients, dialysis patients	29 (3.1%)[b]
Haemophagocytic lymphocytic histiocytosis	6 (0.6%)[c]
Acquired haemophilia A	3 (0.3%)
Other	30 (3.2%)[d]

[a] Clinical reasons identified that lead to mixing studies failing to completely correct, or INR >7.0 or fibrinogen <1.0 g/L.
[b] Typically, isolated prolonged APTT with mildly prolonged APTT on mixing study.
[c] Low fibrinogen or APTT failing to correct.
[d] Majority of patients derived from laboratories where the reporting registrar had no access to clinical records.

Additionally, there were six cases of acquired haemophilia identified during the period of this review. The implementation of mixing studies without an incubation period would not be expected to capture all such cases due to the time and temperature dependent nature of factor VIII antibodies. However, three cases demonstrated a prolonged APTT that failed to correct on the immediate mixing studies.

Table 2 shows identified changes in follow-up test patterns before and after implementation of automated rules.

DISCUSSION

Over a 17-month period, a new laboratory testing and reporting system was implemented. This resulted in a streamlined workflow for the coagulation scientist performing the coagulation studies. There were significantly fewer interruptions to normal workflow required due to reduced requirements of informing clinicians of results, resulting in generalised shorter turnaround times for requested tests, as well as operator savings in time.[4] However, this is balanced by the increased testing of mixing studies, fibrinogen and thrombin time, as seen in Table 2. We have not assessed whether the net effect is an increase or decrease in costs, or perhaps more likely no significant changes in overall costs; nevertheless, we believe the net effect reflects a more targeted solution to follow-up of 'abnormal' coagulation tests.

Table 2 Identified changes in follow up test patterns pre-vs post-implementation of automated rules

	Pre-implementation	Post-implementation
Mixing studies	242	616
Fibrinogen	700	1115
Thrombin time	206	636

Average monthly number of mixing studies, fibrinogen, thrombin time tests performed before, and after, the implementation of the new laboratory testing and reporting system.

Routine warfarin monitoring for stabilised VKA patients is not typically performed within our laboratory, as our setting represents more acute clinical needs. Therefore, INR testing for patients on warfarin with results >7.0 is typically derived from the emergency department, or admitted patients who are unwell or being treated with medications that interact with warfarin. Review of this subset of abnormal results demonstrates that the ordering clinician was aware of the situation and had already implemented appropriate management soon after the result was released.

The six cases of acquired haemophilia A that occurred during the review period were all also detected by the attending clinician based primarily on clinical grounds. In each case, it was clear that the treating clinician had identified a bleeding disorder, and specialist haematology advice had already been sought prior to the rules-based haematology review. The coagulation studies merely acted to confirm the abnormality, and factor level testing occurred without laboratory staff directly prompting the clinicians.

We would argue that, in a setting such as ours, most cases of 'abnormal' coagulation results have no requirement for the clinician to be provided additional advice and guidance for these results.

Firstly, there is an increasing proportion of patients receiving anticoagulation with DOACs.[2,3,18] These patients will typically have coagulation studies above the normal range and mixing studies will not correct. The DOACs have a stereotyped mixing study and thrombin time pattern with the reagents in use in our laboratory. However, without the appropriate clinical information, the interpretation of these results remains speculative and can mimic a variety of other, potentially clinically important, conditions. Most of the patients receiving DOACs did not appear to have a clear reason as to why the basic coagulation studies were initially ordered. Often the coagulation studies were part of routine daily blood tests. Patient history is the best source to determine whether these patients are anticoagulated. Alternatively, DOAC specific drug levels should be used in combination with basic coagulation studies to determine if a DOAC is present.[19]

Secondly, upon retrospective review, most patients appear to have a clear reason to have an abnormal result or a clear clinical reason for which clinician has ordered the test (e.g., heparin infusion, warfarin monitoring, known presence of a DOAC). Unfortunately, this report demonstrates that this clinical reason is still often poorly conveyed to the laboratory and therefore must be determined by review of the electronic medical record. This would be too time-consuming for, and beyond the scope of, the normal activity of laboratory scientists. Approximately 20% of 'abnormal' coagulation results that required haematology registrar review were simply a consequence of a lack of clinical information provided to the laboratory.

Additionally, the results highlight that less than 5% of abnormal coagulation results will require additional specialised coagulation study testing and less than 0.5% of

abnormal coagulation results will lead to alteration in clinical management.

Thus, it is evident that it is possible to reduce the demands on the laboratory scientist without impacting on patient safety.

CONCLUSIONS

In a large tertiary hospital, coagulation studies that are flagged as abnormal are a common occurrence. However, the vast majority of these abnormal results appear to be anticipated by the clinician and do not require further investigation or alterations to clinical management.

Despite the mandatory requirement to provide clinical information on an electronic ordering system, this aspect of clinical-laboratory communication remains poor. This poor communication results in increased demands on the time of laboratory staff who are tasked with either communicating 'abnormal' results to clinicians, even if expected by reasoning of the non-conveyed clinical condition, or performing some follow up action such as reflex testing.

The laboratory process described here anticipates a high volume of abnormal results, and concurrently ensures that the rare unexpected and potentially clinically important abnormal results receive further evaluation. Immediate reflex second-line coagulation testing and medical review of abnormal mixing studies and hypofibrinogenaemia ensures that patient safety is not compromised. Overall this improves laboratory work-flow by reducing the demands on the coagulation scientist. We are currently revising the rules set in response to the findings of this audit investigation.

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