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ORIGINAL PAPER

Serologic assessments in acute transfusion reactions: practices and yields

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Vox Sanguinis

Background & Objectives Serologic testing after transfusion reactions (TRs) aims to find accountable immune haemolytic incompatibility. Our hospital policies recommend serologic testing in all TR, except for low-risk fevers (subclinical temperature <39°C) or uncomplicated allergic reactions. Assessing compliance with these guidelines and serologic testing yields may provide insights on quality of practice and value.

Materials & Methods Interrogation of two haemovigilance databases identified all possible-to-definite TR over a 4-year period (2013–2016) at four academic hospitals. We reviewed the performance and outcome of serologic testing by site, year, reaction type, implicated product and service.

Results Serologic testing occurred in 769 (55%) of 1408 referrals, with 1153 (82%) compliant with guidelines. Similar proportions deviated to overtesting (85/550 [15%]) and undertesting (174/858 [20%]), with undertesting seen most often in atypical TR. Overall, 30 (4.4%) of 769 cases had a new finding, but only 2 (0.3%) reflected host-derived antibodies. Overall, the number needed to test to discover an unexpected allospecificity was 385, or 253 if limited to high-risk fevers. Reaction- and product-specific yields ranged from 0% to 48%. The yield in complicated allergic reactions was low at 2%, constituting only predictable passive isohaemagglutinin(s) in retrospect. Investigated IVIG TR accounted for most of this cohort's signal by passive isohaemagglutinins in 48%.

Conclusion The performance of post-TR serologic testing revealed practice gaps and expected context-specific yields. Tailored serologic testing (i.e. indirect antiglobulin tests for alloantibodies in post-RBC/high-risk febrile reactions, ± isoagglutinin-focused tests after IVIG or ABO-minor-mismatched platelets) may improve value and liberate resources for other unmet needs in TR investigation.

Key words: transfusion reaction, serologic testing.

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Introduction

Transfusion reactions (TRs) occur in 1–10% of transfusion encounters [1,2]. While most are minor (as seen in our passive system's reporting rate of 0·4–0·3%), [3] serious events (and those with fever) raise concerns relating to a new or missed immune haemolytic incompatibility. Laboratory findings may thus explain recipient harm and justify higher-fidelity RBC antigen matching.

Serologic testing of 'transfusion reaction samples' includes the performance (and pre-transfusion comparison) of grouping (ABO, RHD type) and red cell antibody screening (indirect antiglobulin testing [IAT]), with a post-transfusion direct Coombs test (direct antiglobulin testing [DAT]). Locally, such serologic testing costs roughly $100 CAD, not including the cost of additional reflex tests (e.g. elutions, panel investigations or IAT crossmatching) and medical review (~$50 CAD) [4].

Locally, the approach to a TR is to perform serologic testing, with the exception of those alterations assumed to have a low pre-test probability of incompatibility or transfusion-transmitted sepsis [5]. serologic testing exceptions comprise the low-risk fever (temperature maximum [Tmax] <39°C with no other symptoms or signs) and uncomplicated allergic reaction (urticarial rash in the absence of other findings) [6]. In a high-volume setting, the extent to which serologic testing occurs unnecessarily (beyond policy), or insufficiently (despite policy), and the final yield of these results (in general or by reaction type), is not known.

The primary objectives of this retrospective audit (of prospectively acquired haemovigilance data) were to determine non-compliance frequency (of overtesting and undertesting) and to describe the findings of serologic testing (overall and by reaction presentation). These data may drive refinements in serologic testing approaches by TR type.

Methods

Institutions involved and populations served

Four academic hospitals were included in the analysis, with all sites receiving blood products from Canadian Blood Services; components included red blood cells (RBC), platelets (PLT [single donor apheresis or buffy coat pools]), plasma and cryoprecipitates. Cellular components were pre-storage-leucoreduced. Derivatives included IVIG, albumin and coagulation factor concentrates (plasma-derived or recombinant).

Site A (Sunnybrook Health Sciences Centre, 627 beds) is a large general hospital with cancer care, trauma, obstetrics and surgical services. Site B (Toronto General Hospital, 457 beds) is a tertiary care facility with transfusions supporting an inherited RBC disorder programme, apheresis and surgical services (complex cardiac and multi-organ transplantation). Site C (Toronto Western Hospital, 280 beds) is a general hospital specializing in orthopaedic and neurosurgery. Site D (Princess Margaret Cancer Centre, 129 beds) is a specialized cancer centre with a cell therapy programme.

Data sources

Two passive haemovigilance databases contained information on all referrals for a possible TR at each of four teaching hospitals in Toronto, Canada, between 1 January 2013 and 31 December 2016. A transfusion safety officer investigated referrals and reported on TR in accordance with requirements of the Transfusion-Transmitted Injuries Surveillance System (Public Health Agency of Canada) [7]. Data collected included the clinical history of the patient; the date, time and place of the reaction; clinical/laboratory results (including VS); and information about the implicated products. The type and severity of the reaction; relation of the adverse event to the transfusion (i.e. certainty of diagnosis/product imputability); and the results of serologic testing were also collected. Research Ethics Board approval was obtained for each site (#148-2017 at Sunnybrook Health Sciences Centre [site A], and CAPCR ID# 17-5235 at University Health Network [sites B-D]), with a data transfer/sharing agreement (#2017-0339). Denominator (components-issued) transfusion activity in 2016 was 16 575 at site A and 67 033 at sites B-D.

Serologic Testing

At site A, both the DAT (manual [polyspecific/monospecific IgG/monospecific C3d]) and IAT (automated [monospecific IgG]) were performed in gel (with anti-human globulins [AHGs], reagent red cells and gel columns from Bio-Rad Canada [Montreal, QC, Canada]). At sites B-D, the DAT (manual [polyspecific/monospecific IgG/monospecific C3d]) was performed in tube, while the IAT (usually automated [monospecific IgG]) was performed in solid phase (with AHGs, reagent red cells, and Capture platform from Immucor [Peachtree Corners, GA, USA]). IAT panels were subject to comparative expansion in gel (automated or manual, Bio-Rad reagents) and/or solid phase (automated, Immucor reagents) and/or tube (manual, Immucor reagents). All sites used a 2-cell screen, and elution studies (manual) used Gamma® ELU-KIT® II (Immucor). Eluates were performed on every positive DAT, and despite a negative DAT if there was evidence for haemolysis.

Strengthened reactions were those which had increased by at least one semi-quantitative grade in at least one platform for the involved target antigen. Reagent red cells were group O, unless type-specific red cells were used to examine for isohaemagglutinins (in IVIG-associated reactions or in platelets with plasma [minor] incompatibility). Techniques were constant over the study period.

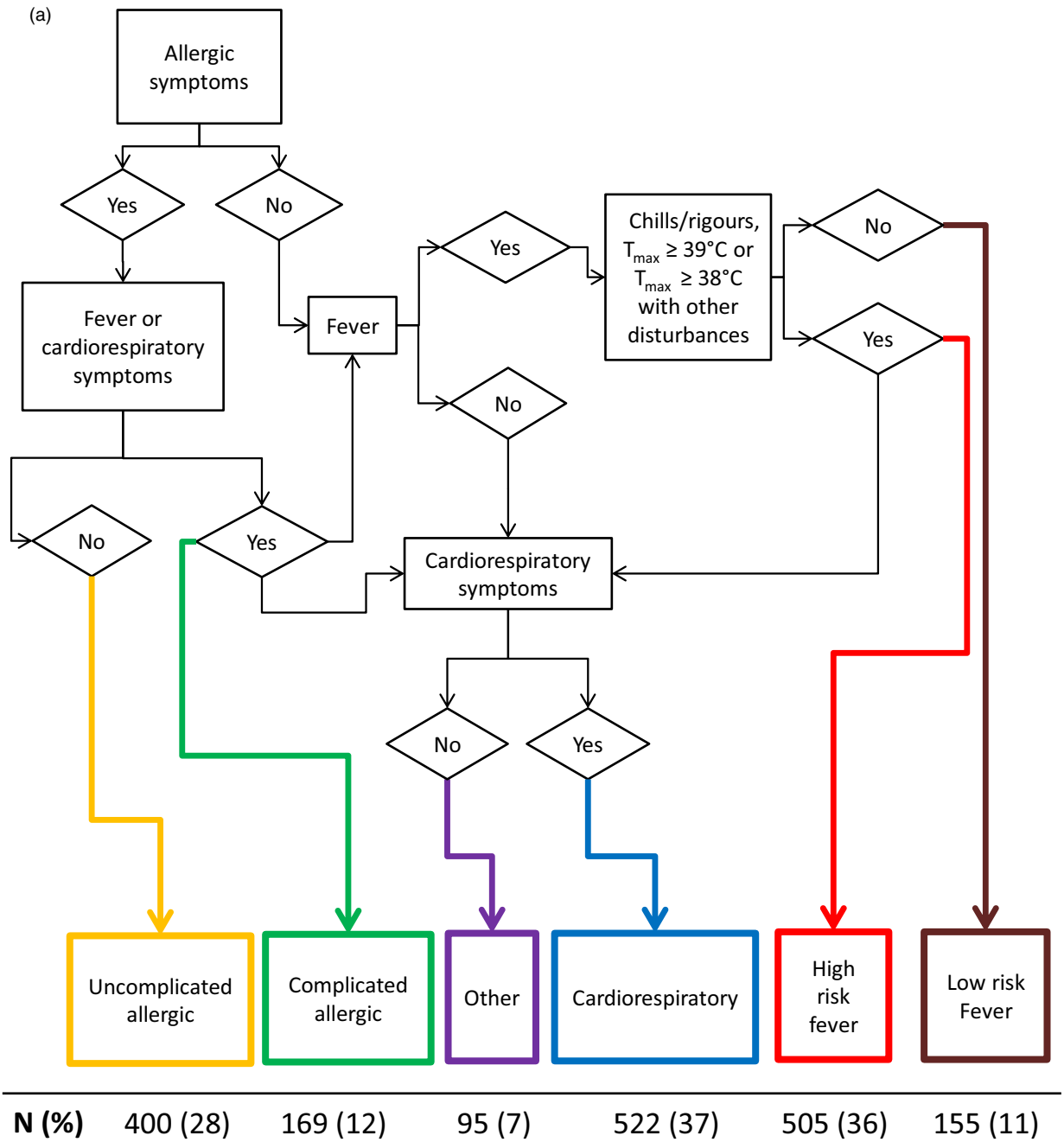


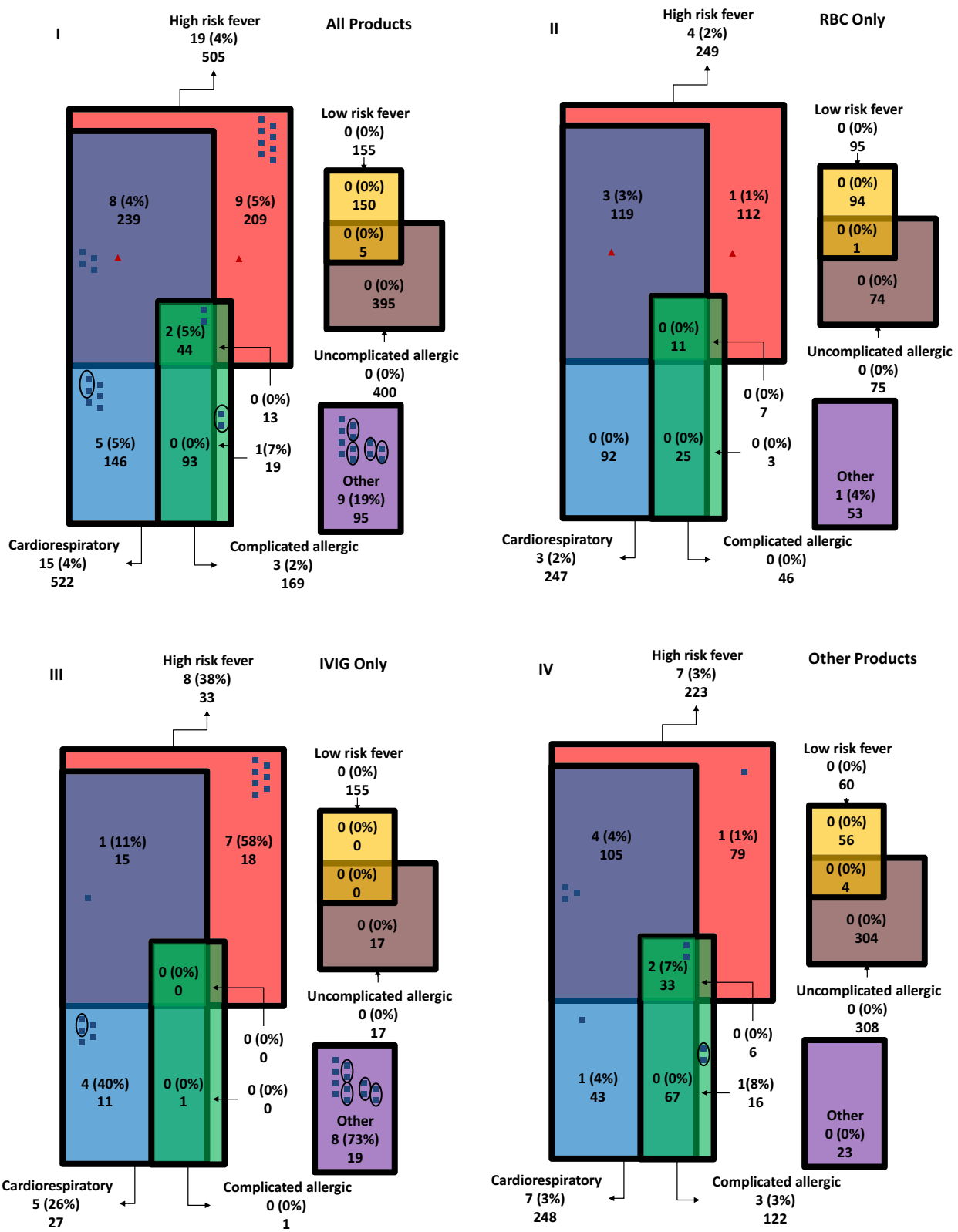
Fig. 1 Classification and overlap of reaction presentations. (a) Reaction presentations and classification process. (Some presentations qualify under multiple categories and therefore sum to >100%.). (b) Reaction presentations and overlaps. The number of reactions in a category (denominator) is shown with reactions with positive serology (numerator) and corresponding yield, *N* (%). ■ acquired isoagglutinin (oval reflects anti-A + anti-B found in a case). ▲ non-ABO antibody. Individual patients with a positive yield can have more than one antibody discovery. (I) All products, (II) RBC only, (III) IVIG only, (IV) Other products (e.g. platelets or RBC in combination with other products). [Colour figure can be viewed at wileyonlinelibrary.com]

Characterization of reaction events and data definitions

Reaction presentations were pragmatically classified according to presenting alterations. Categories included fever (high-risk versus low-risk), allergic (complicated versus uncomplicated), cardiorespiratory or 'other'.

A high-risk fever encompassed any temperature increase by ≥1°C to ≥39°C, and/or the presence of either chills or rigours, and/or a temperature increase to ≥38°C

(b)



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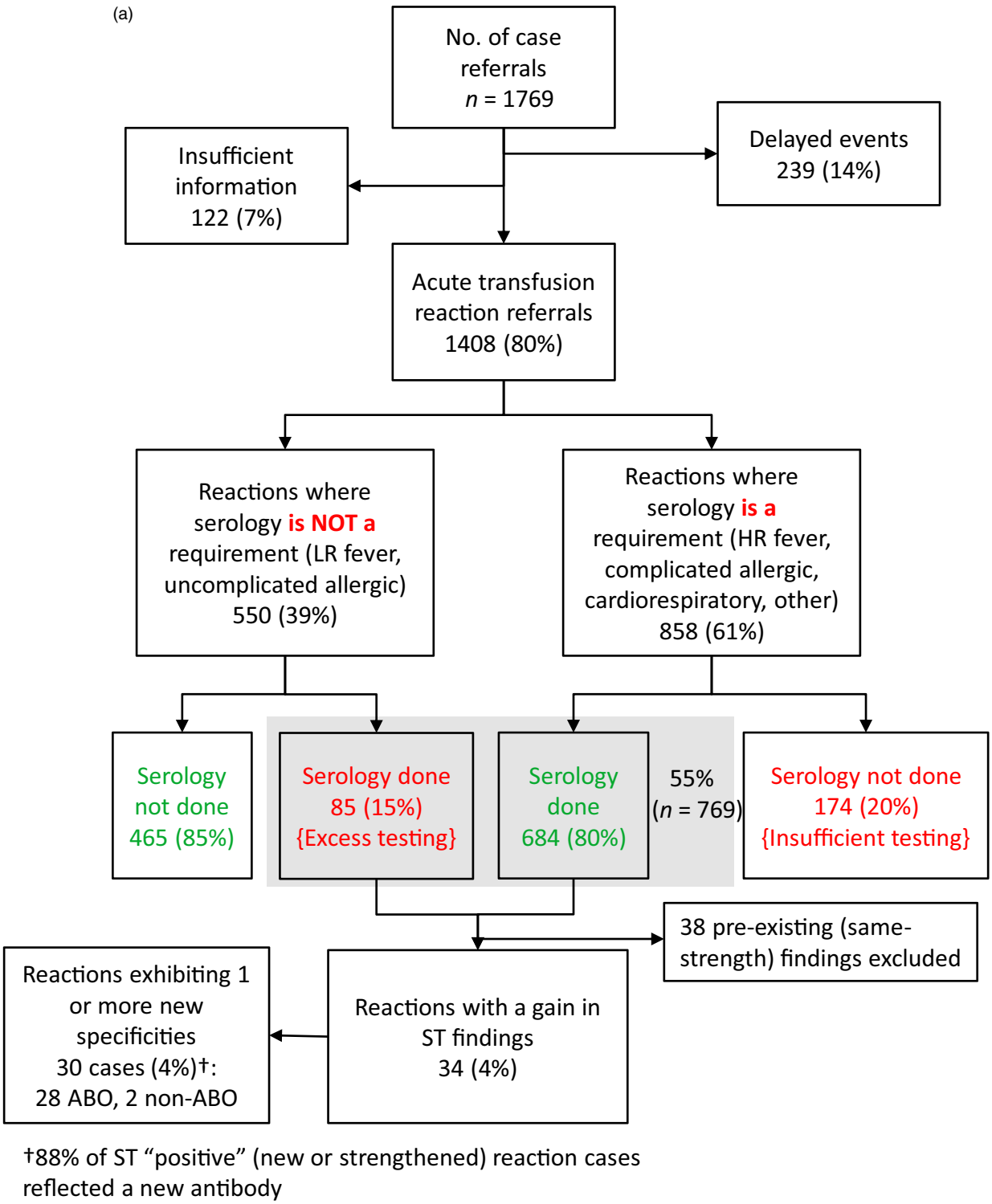

Fig. 2 Process flow diagram of serology requirements and acute reaction capture. (a) Reaction sorting according to serologic testing expectations. (b) Disturbances according to acute and delayed presentations. (Due to overlaps, sums exceed 100%). [Colour figure can be viewed at wileyonlinelibrary.com]

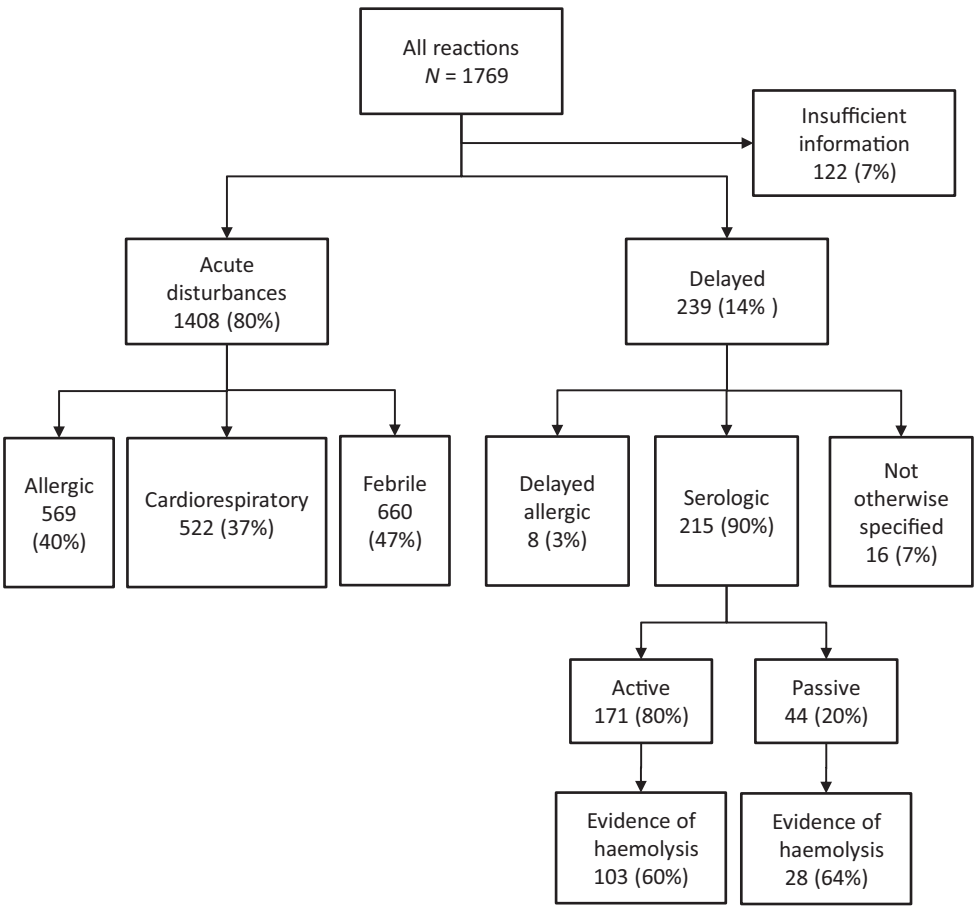

Fig. 2 (Continued)

with any vital sign (VS) instability or other non-allergic symptoms (including but not limited to dyspnoea, wheezing, pain, bleeding, haematuria, oliguria, haemoglobinuria, nausea or vomiting). A low-risk fever constituted any temperature increase by ≥1°C to reach a range of 38–38·9°C, as long as this change occurred without any other new changes.

Cardiorespiratory reactions were those with dyspnoea, wheezing/crackles, chest pain and/or VS instability. VS instability was defined by significant deviations from baseline ranges, that is a rise or fall in heart rate (HR) by Δ≥10 from normal range (60–100), a rise or fall in systolic blood pressure (SBP) by ≥30 mm Hg from normal range (90/40–140/90), significant shifts from normal respiratory rate (12–20), or SpO_2 reduction by at least Δ5% to ≤90%. Clinician discretion was applied to the determination of VS instability to limit overdiagnosis of reactions, which merely represented consistent shifts within the patient's foregoing variation pattern, and to prevent underdiagnosis of reactions where changes may have been significant despite aforementioned definitions (e.g. SBP 89 to 60 by −Δ29).

An uncomplicated allergic reaction was defined as any rash, hive or other allergic symptom (whatever the total body surface area percentage involved), without any VS disturbances. A complicated allergic reaction was defined as any allergic symptoms in conjunction with VS instability, cardiorespiratory symptoms or airway anatomy oedema.

Policies, irrespective of involved product, require serologic testing in all serious or atypical reactions. Serologic testing is waived in low-risk fever or uncomplicated allergic reactions, although deviations may occur by choice of the bedside healthcare team.

As reaction presentations sometimes involved more than one disturbance, a stratification rule (based on dominant presenting features rather than the final diagnosis) was created for analysis (Fig. 1a). Allergic reactions were classified as complex if they involved febrile and/or respiratory features, while still contributing to the analysis of febrile and respiratory subgroups if applicable. Febrile reactions with cardiorespiratory disturbances contributed to both reaction subgroups for analysis. Due to the frequency of overlap cases (Fig 1b), the analysis began with

all cases in the cohort, rather than the sum of cases in all subgroups (otherwise exceeding 100%).

If an atypical reaction did not meet any aforementioned criteria, it was classified as 'other'. Examples included atypical presentations involving pain (e.g. headaches), ischaemia/inflammation (e.g. query associated vascular thrombotic events) and seizures/ sensorium change/loss of consciousness.

Serologic testing was deemed guideline-compliant if performed when advised, or not performed when not required (Fig. 2a); deviations were thus overtesting (ST when not required) or undertesting (ST not done when required). Serologic testing was positive if the post-transfusion DAT or IAT was positive, and the pre-transfusion test was negative for the deduced specificity, or a pre-transfusion specimen did not exist (e.g. prior to IVIG). Thus, a positive yield reflected a new antibody distinct from the previous repertoire.

When pre- and post-TR antibody screens were negative, with a positive post-reaction DAT but a negative eluate (including towards non-O RBCs in TR involving relevant isohaemagglutinins), the result was considered unrevealing, that is implying a possible drug-induced antibody or non-specific antibody binding.

If an antibody against a low-frequency antigen was suspected by parameters of haemolysis, then IAT re-cross-matches with segments of the implicated units were performed with the post-TR specimen.

The serologic testing yield was calculated by dividing the number of positive serologic testing by the number of reactions that underwent serologic testing for the group of interest.

To prevent asymptomatic or delayed screening discoveries from artificially inflating serologic testing yields, all serologic changes incidentally identified were excluded (Fig. 2b), as the emphasis in this study was yield in acute TR. Reactions were excluded if lacking sufficient information for classification. Each case was previously concluded by transfusion medicine physician review (YL, LL, JP, JC, CC-G) in the electronic health record.

Estimated cost of ST

The cost of serologic testing investigation of a TR (materials and labour) was computed at $98 CAD. This did not include added costs of identifying antibodies in specificity determinations. Estimates for activity-based serologic testing costs vary from $114 USD [8] to €110 EUR [9].

Statistical analysis

Descriptive statistics were reported as means with standard deviations or as medians with interquartile ranges for skewed data. An unpaired *t*-test was used to calculate differences in serologic testing performance rates and yields. All *P* values <0·05 were considered statistically significant. Calculations were performed in Excel (2016; Microsoft Corporation) and GraphPad Prism (version 6.04 for Windows; GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

Results

There were 1769 TR referrals during the 4-year study period. We excluded 239 (14%) events due to delayed presentations, while another 122 (7%) reactions were excluded as they lacked sufficient information to determine their acuity. Of the 239 delayed reactions, 215 (90%) were delayed serologic transfusion reactions, and 131 (61%) of these had some evidence for haemolysis (Fig. 2b). A total of 1408 acute reaction referrals remained, of which 1342 were deemed to be transfusion-attributable (i.e. imputability possibly to definitely related to transfusion). In 66 cases, the referral was regarded as transfusion-unrelated (Fig. S1).

Table 1 Patient history and demographics

Parameter	All Sites
No. of referrals	1769
No. of acute reaction referrals	1408
2013	336
2014	351
2015	366
2016	355
Affected patients	1115
Confirmed reactions	1342
Age, years (mean ± SD)	56 ± 17
Female, *N* (%)	685 (49)
History of pregnancy, *N* (%) (in females)	387 (56)
History of transfusion, *N* (%)	1190 (85)
History of transfusion reaction, *N* (%)[a]	346 (30)
Product type, *N* (%)	
RBC only	591 (42)
PLT only	405 (29)
RBC or PLT or both	1208 (86)
IVIG only	81 (6)
Other products[b]	119 (8)
Patient location *N* (%)	
Outpatient	615 (44)
MSW	579 (41)
ICU	138 (10)
ER	54 (4)
OR	22 (2)

[a]No history data from site A.

[b]Other products: plasma, cryoprecipitate, albumin or combinations of products other than RBC and PLT.

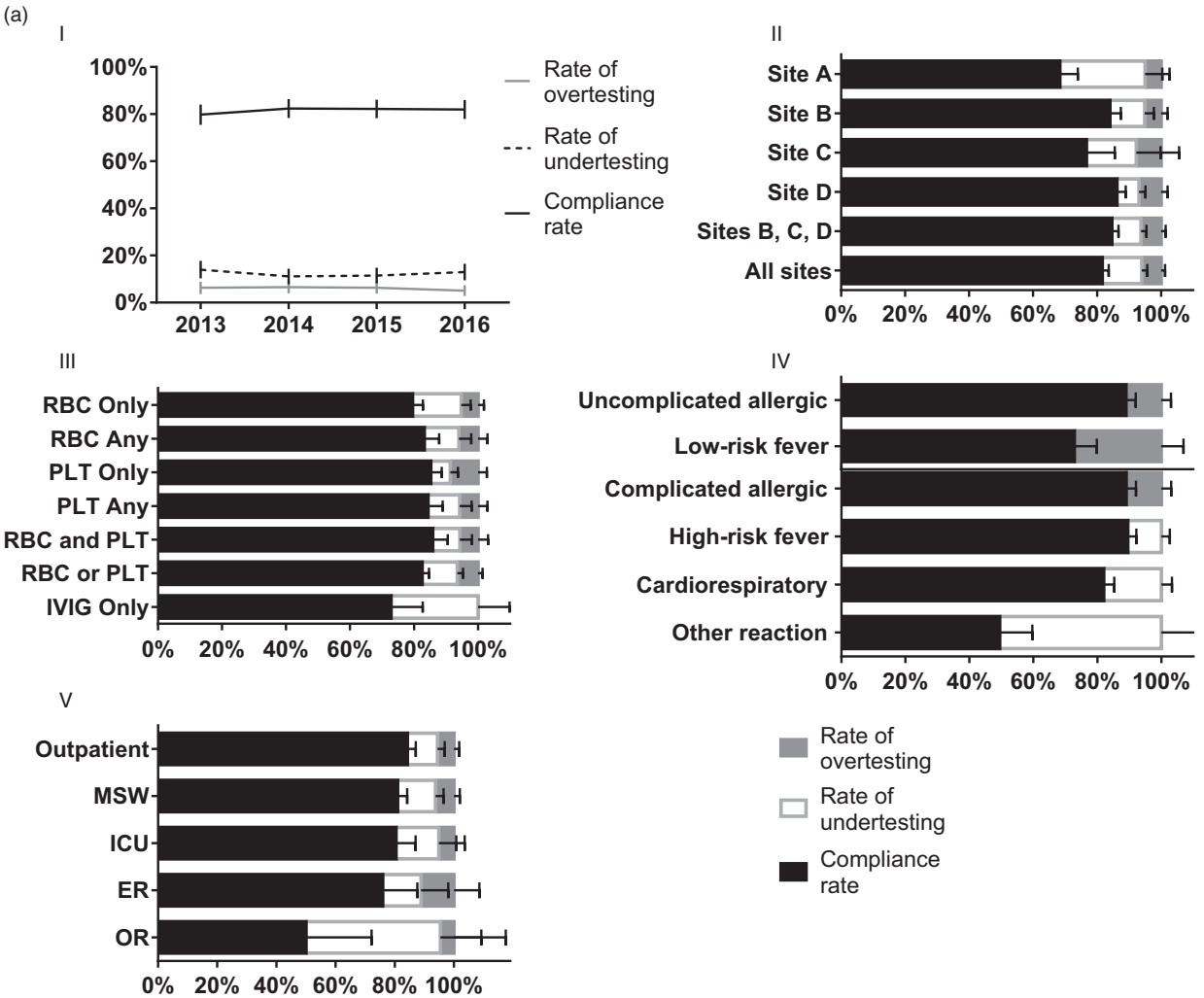

Fig. 3 Serology testing and yields. (a) Compliance of serologic testing with policy by (I) year, (II) site, (III) product, (IV) reaction presentation and (V) location. (b) Frequency of serologic testing and corresponding yield by (I) year, (II) site, (III) product, (IV) reaction presentation and (V) location. Error bars represent 95% confidence interval. MSW: medical/surgical wards, ICU: intensive care units, ER: emergency rooms, OR: operating rooms.

Of the acute TR referrals, 1115 unique patients experienced at least one TR in the audit period. The number of TR referrals was similar over time (range: 336–366 per year). Half (49%) of patients were female, and 56% had been previously pregnant. Most (85%) patients had prior transfusion(s), and 30% of referrals occurred in patients with a prior TR. Most (85% of) TR occurred on medical/surgical wards or outpatient transfusion clinics. Cellular components (RBC or PLT) were most commonly implicated at 86% (Table 1).

Characterization of reactions

TRs were classified by the presenting disturbance (Fig. 1a). Uncomplicated allergic, high-risk fever and cardiorespiratory presentations were commonest, with substantial (38%) overlap between high-risk fever and cardiorespiratory events (Fig. 1b and Fig. S2). Only 23% of febrile reactions qualified as low-risk, compared with 70% of allergic reactions being uncomplicated (Fig 1a). Final reaction diagnoses for the cardinal TR disturbances are shown in Fig. S2.

Primary objective: application of serologic testing (quality of practice)

Policy advised serologic testing in 858 cases (61%) (Fig. 2a), whereas its conduct occurred in 769 (55%). Compliance with serologic testing policy (not testing when not indicated, and testing when indicated) occurred in 1149 (82%) of TR. Serologic testing behaviours did not change year to year (Fig. 3a). Serologic testing was

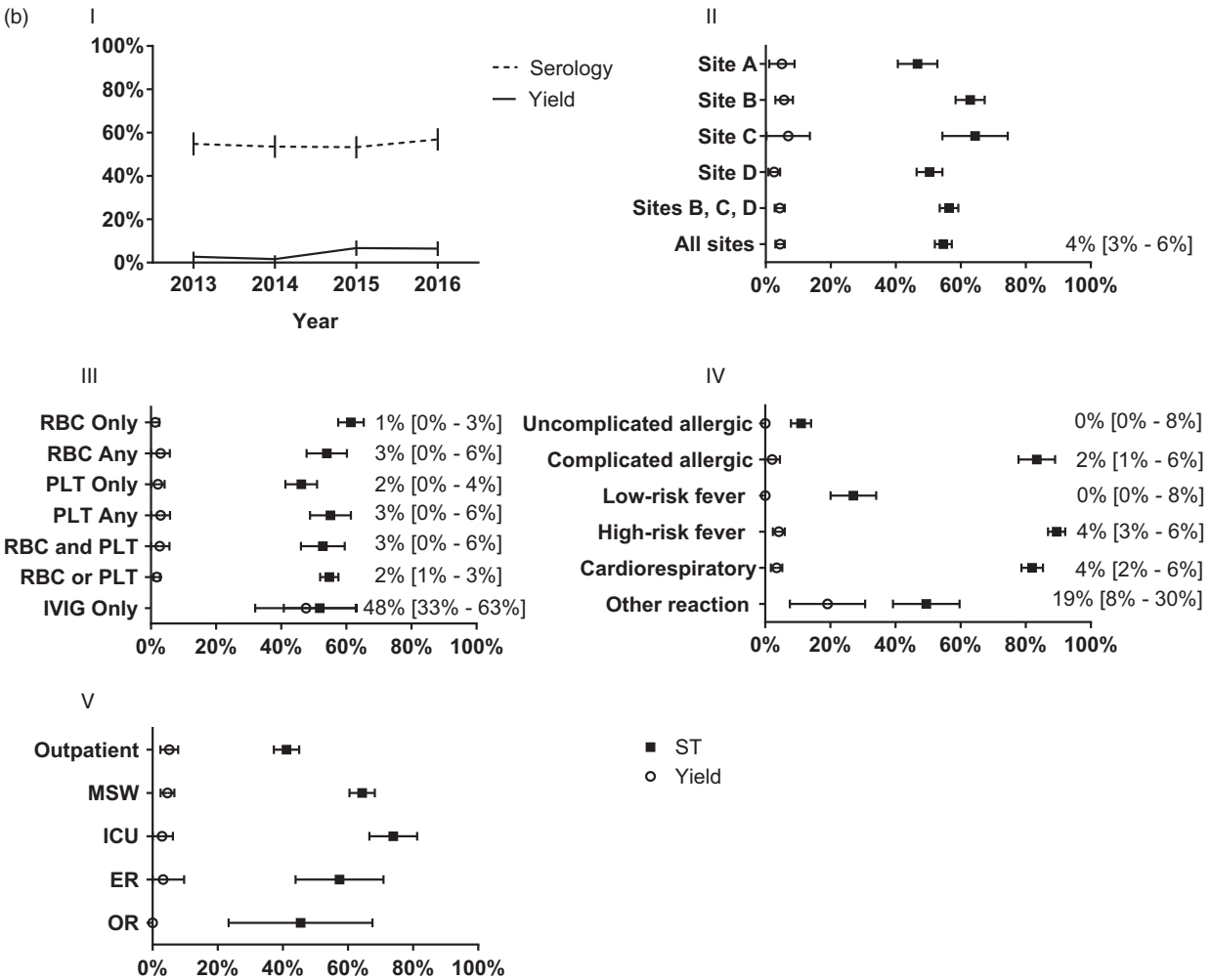

Fig. 3 (Continued)

performed less frequently at site A compared with sites B-D (47% vs. 56%; $P < 0{\cdot}01$, Fig. 3b) and was also less compliant (68% vs. 85%; $P < 0{\cdot}0001$). In 85 cases, testing was performed unnecessarily, and none of these cases yielded a positive result (95% confidence interval [CI]: 0–5%). Of presentations where testing was not required, low-risk fevers were the most frequently overtested entity (27%), while the highest compliance rate was observed in high-risk fever (for testing) and uncomplicated allergic reactions (for not testing) at 90% and 89%, respectively. The highest rates of undertesting were found in reactions involving IVIG (27%) and in 'other' atypical reactions (51%).

Secondary objectives: serologic testing yields

In serologic testing cases, 4% of (34) investigations generated newly positive findings, while 38 baseline-sero-positive cases showed no change (Fig. 2a). Of 34 cases with serologic gains, one or more new antibodies were discovered in 88% (30 cases), with 28 cases exhibiting one or more acquired isoagglutinins, and two cases revealing a new non-ABO specificity that had been missed on prior screens (Sc2, AnWj). Four of 34 cases merely exhibited strengthened reactions of previous antibodies (passive anti-D [after re-administration of Rh immune globulin, accompanied by acute haemolysis], anti-E despite E-RBC and auto-anti-AnWj in two successive acute haemolytic transfusion reactions following transfusion of AnWj + RBCs when AnWj– RBCs were not available).

Serologic testing yields varied widely by reaction presentation and product type. High-risk fever (4% [95% CI: 2–6%]) and cardiorespiratory reactions (4% [95% CI 2–5%]) had higher yields compared with complicated allergic reactions (2% [0–5%]), while the yield for 'other' (atypical) reactions was highest (19% [8–30%]) (Fig. 3b, Table S1). Despite the yields of the latter, reactions with

Table 2 Antibodies denominator reflects the number of acute reaction referrals or applicable subpopulation

	No. of antibodies identified, *N*	Patients with antibody identified, *N* (%)	Patients with ABO antibody, *N* (%)	Patients with non-ABO antibody, *N* (%)
Reaction classification				
Low-risk fever	0	0 (0)	0 (0)	0 (0)
Uncomplicated allergic	0	0 (0)	0 (0)	0 (0)
Complicated allergic	4	3 (2)	3 (2)	0 (0)
High-risk fever	16	16 (4)	14 (3)	2 (0)
Cardiorespiratory	13	12 (3)	11 (3)	1 (0)
Other reaction	12	8 (17)	8 (17)	0 (0)
Product type				
RBC	2	2 (1)	0 (0)	2 (1)
PLT	5	4 (2)	4 (2)	0 (0)
RBC or PLT or both	10	9 (1)	7 (1)	2 (0)
IVIG	25	20 (48)	20 (48)	0 (0)
Other products	1	1 (2)	1 (2)	0 (0)
Reaction severity				
Minor	7	6 (2)	6 (2)	0 (0)
Moderate	23	19 (7)	17 (6)	2 (1)
Severe	6	5 (4)	5 (4)	0 (0)
Life-threatening	0	0 (0)	0 (0)	0 (0)
Fatal	0	0 (0)	0 (0)	0 (0)
All reactions	36	30 (4)	28 (4)	2 (0)

Table 3 Identity of positive serologic discoveries

Antibody (anti-)	No. identified on DAT/eluate	No. identified on screen
A	25	0
B	9	0
Jka	0	0
K	0	0
Lua	0	0
Sc2	0	1
P1	0	0
AnWj	0	1
Total	34	2

high-risk fever or cardiorespiratory symptoms nevertheless contributed most serologic testing discoveries at 61% of (22/36) antibodies (Fig. 1b, Table 2).

However, all findings in the complicated allergic reactions (involving three recipients) and in the 'other' category (involving nine recipients) solely reflected acquired ABO isohaemagglutinins rather than host-derived antibodies. After subtracting isohaemagglutinins, the rank order for informative (non-isohaemagglutinin) discoveries was owing to a patient with high-risk fever (one case, targeting Sc2) [10] and a reaction with both high-risk fever and cardiorespiratory features (one case, targeting AnWj, in its first recognition).

Product-specific yields showed that reactions associated with RBCs, PLT or both were comparable at around 2% [1–3%], although serologic testing was performed significantly more often for reactions involving RBC when compared with reactions involving only PLT (61% vs. 46%; $P < 0{\cdot}001$). Of interest to dispensaries of IVIG, the highest product-specific serologic testing yield was for this product, at 48% of IVIG TR (or 70% of the non-O recipients in this subset). A disproportion (25/36 or 69%) of all newly observed antibodies were passively acquired isohaemagglutinins, even though IVIG was implicated in only 6% of TR (Tables 1 and 2). Twelve of 20 (60%) IVIG-sensitized patients experienced some degree of delayed haemolysis in post-TR follow-up.

In contrast, all new antibodies identified after an acute reaction to RBC alone were against non-ABO antigens and despite negative pre-transfusion antibody screens. In the anti-Sc2, neither antibody screening cell carried the low-frequency Sc2 target, while in the high-frequency auto-anti-AnWj, the pre-exposure plasma did not contain a detectable titre of the antibody (Table 3).

Antibody discoveries occurred more often with the DAT (or its blind eluate [in 8 of the 29 eluate-positive results]), than with the IAT (29:7), with minimal overlap (two cases) (Fig. S3). This margin was due to ABO isohaemagglutinins (in 28 of the 29 DAT + or 30 serologic testing+ cases). Phase findings associated predictably with

the implicated product, in that all DAT discoveries followed TR with IVIG, RhIg or plasma-incompatible platelets. After subtracting predictable passive antibodies, reactions with yields from the IAT phase followed high-risk reactions involving RBC.

Discussion

Serologic testing (ST) is central to detecting immune haemolytic incompatibility in transfusion reactions (TRs). However, non-compliance with testing algorithms – both in overtesting and in undertesting – can lead to increased workload and costs (for the former), or to the failure to identify and prevent incompatibility-related reactions (in the latter). In this 4-year audit at four hospitals, serologic testing was done in over half of TR, but was left unexecuted in a fifth of test-worthy cases. When performed, serologic testing rarely yielded positive (informative or impactful) findings.

The freedom to waive serologic testing in low-risk fevers and in uncomplicated allergic reactions [6] (both comprising the theoretical majority of reactions) has been justified to both reduce the burden of low-yield tests and to remove disincentives to reporting. As there were no serologic testing discoveries from low-risk events in the windows of overtesting, it is difficult to argue for its expansion.

This examination of referrals was pragmatic, as it was based on presenting features soon after a product exposure (rather than on the deduced final diagnosis or delayed TR). High-risk referrals (high-risk fever, cardiorespiratory, complicated allergic or 'other') outnumbered low-risk events (858 vs. 550). This likely reflected reporting bias, as less serious events may be deliberately underreported to reduce disruptions by unwanted investigations. This phenomenon was reported in our study of febrile non-haemolytic transfusion reactions (FNHTR), with high-risk fevers enriched among FNHTRs [11]. The absolute volume of high-risk reactions drives a high activity level and costs for the transfusion laboratory, despite pre-existing policies to minimize overtesting. As a result, an assessment of the differential yields of tests (according to presentations and products) was desired, lest it reveal that certain contexts are more or less informative than others.

This analysis presented both reaction- and product-specific serologic testing yields, as well as the source and specificities of the antibodies discovered. Given that high-risk fevers, cardiorespiratory changes, and reactions classified in the 'other' category had the highest serologic testing yields, there are no grounds for abandoning serologic testing in these cases. However, other reaction presentations may warrant less attention, while certain products (or product–recipient combinations) may deserve tailored ST.

Complicated allergic reactions had the lowest yield among serious TR and null yields if passively acquired (predictable) IVIG-related antibodies were excluded, thereby arguing against comprehensive serologic testing in allergic presentations. Indeed, RBC-specific antibodies are not intuitively involved in allergic–anaphylactic reactions, unless IAT investigations reveal Chido [12] or Rodgers [13] antibodies. However, neither of these specificities were found in allergic (or non-allergic) reaction cases in this audit, nor in a larger review of the association of these specificities with allergic outcomes (with 121 C4-sensitized individuals found in 22 years [1993–2014] at centres B-D, 65 of whom were transfused at least once, with none having allergic reactions) [14].

In the analysis of product-specific yields, IVIG TR had the highest rate of serologic testing findings compared with other products (by an order of magnitude [48% vs. 1–3%]). All findings occurred in non-O reactors (for a 70% yield therein). This rate was higher than the rate of isohaemagglutinin-related DAT positivity in a parallel prospective study of non-O recipients of IVIG (with subjects cross-captured only if experiencing acute reactions) [15].

If isohaemagglutinin detection and feedback reporting are valued, then all reactions to IVIG in non-O recipients, irrespective of the clinical presentation and severity, may merit the DAT, while the value of grouping and re-screening is yet to be seen. Given that positive serologic testing findings are so predictable (or falsely negative otherwise), efforts may focus on haemolysis monitoring instead. If isohaemagglutinins and/or haemolysis are found, patients may then be committed to group O RBCs (if consequently anaemic enough to require transfusion support) until isohaemagglutinins clear.

To our knowledge, this is the first study to provide figures on the applied performance and yields of TR serologic testing in a real-life, multi-institutional setting with multiple serologic testing platforms. However, this study is not without its limitations. The sample size, despite data pooling over years across multiple hospitals, nevertheless generated similar serologic testing yields with overlapping confidence intervals despite some hypothesis-generating hierarchies.

Data to assess the yield of low-risk reactions were limited as a direct result of the guidelines that dissuade testing; as such, the only available data were those that existed by non-compliance. Larger data sets are needed to eliminate possible beta errors, as the absence of findings in the overtested cohort of 85 low-risk reaction profiles maintains a margin of possible discovery as high as 5%, unless a cohort ten times the size redresses the odds to

<0·5%. At the same time, 'low-risk' cases subjected to serologic testing may have engendered greater concern (and thus the odds of a true immune injury), although yields were nevertheless low. The risk of dismissing severely immunocompromised patients (who are theoretically limited in their capacity to manifest higher-grade pyrexia) is a concern, but we have previously found this subpopulation to nevertheless be disproportionately represented in ST-qualifying fevers by virtue of rigours or other symptoms and signs [4].

Our haemovigilance databases, founded on passive reporting, may not reflect the yields that prospective reaction cohorts with uniformly enforced and common testing standards might generate. Low-risk reactions may have greater yields than we have ascertained, while the same may apply to those of our higher-risk reactions where serologic testing was not performed despite indications. Non-performance of serologic testing may reflect care gaps or technically prohibitive case features (e.g. instability with care escalation and location change), which may in turn reflect cases with missed yields. As such, these numbers err on the side of underestimation. Obstacles to compliance were not elucidated in this cohort.

Finally, while we and others have instituted similar guidelines on TR ST, we recognize that other hospitals may already have relaxed their policies (with scepticism on the informativeness of eluate testing as well) [16]. Continuing with the cost of comprehensive serologic testing might only be justified in environments with low caseloads or resistance to nuanced algorithms. In this study, the number of reaction cases needed to test (NNT) for serologic testing to find a clinically significant non-ABO host-derived RBC antibody was 385 (or>$38 500 in system expenses per discovery). In these cases, the patient's next crossmatch sample submissions may have revealed the specificity regardless. The cost per finding is also likely underestimated because expenses are higher when yields are positive and panel investigations ensue. The high volume of TR (and downstream costs of definitively investigating reactivities internally or at reference locations) is further additive.

In spite of these limitations, our study offers rough and rank-order estimates on serologic testing yields to guide blood banks if their capacity and/or budgetary resources are constrained. Deferring serologic testing in allergic reactions can re-focus a costly workload to those cases where the pre-test probability of an actionable finding is highest, while conversely reducing the burden of indeterminate results or false positives from the effects of high-sensitivity platforms in low pre-test probability cases. Taken together, RBC reactions justify re-grouping and screening, while IVIG or plasma-mismatched platelets may trigger the DAT and/or tests for haemolysis with high-risk (non-allergic) features. Without having yet examined the implementation of streamlined policies, these changes may be easier said than done for saving workload while preserving discovery rates.

In conclusion, we have quantified serologic testing workloads and policy compliance gaps, showing that there is room for improvement. Studies are warranted on barriers to completing serologic testing when indicated for actionable yields. Until larger and more comprehensive cohorts on post-TR serologic testing yields are characterized, this practical multi-institutional audit provides an approach and rank-order estimates of overall, reaction-specific and product-associated serologic testing yields. Sites may therefore modify their policies on serologic testing in acute TR in accordance with their available resources and views on acceptable risks.

Conflicts of Interest

None.

References

1 Kaufman RM, Assmann SF, Triulzi DJ, *et al.*: Transfusion-related adverse events in the Platelet Dose study. *Transfusion* 2015; **55**:144–153

2 Hendrickson JE, Roubinian NH, Chowdhury D, *et al.*: Incidence of transfusion reactions: a multicenter study utilizing systematic active surveillance and expert adjudication. *Transfusion* 2016; **56**:2587–2596

3 St Bernard R, Yan M, Ning S, *et al.*: Sustained and significant increase in reporting of transfusion reactions with the implementation of an electronic reporting system. *Transfusion* 2016; **56**:1247–1248

4 Cohen R, Escorcia A, Tasmin F, *et al.*: Feeling the burn: the significant burden of febrile nonhemolytic transfusion reactions. *Transfusion* 2017; **57**:1674–1683

5 Hong H, Xiao W, Lazarus HM, et al.: Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance. *Blood* 2016; **127**:496–502

6 TTISS-On: TTISS transfusion reaction charts. 2017. https://ttiss.mcmaster.ca/wp-content/uploads/2017/08/Transfusion-Reaction-Chart-V_2.2-Pocket-and-Poster.pdf [Last accessed 13 November 2018]

7 TTISS-On: Ontario guide for reporting transfusion reactions. 2018. https://mctr.mcmaster.ca/surveys/?s=ELF8R8JPYE [Last accessed 13 November 2018]

8 Mazonson P, Efrusy M, Santas C, *et al.*: The HI-STAR study: resource utilization and costs associated with serologic testing for antibody-positive patients at four United States medical centers. *Transfusion* 2014; **54**:271–277
9 Janssen MP, Tilborgh AJW, Vooght KMK, *et al.*: Direct costs of transfusion reactions – an expert judgement approach. *Vox Sang* 2018; **113**:143–151
10 Lemay AS, Tong TN, Branch DR, *et al.*: The first case of severe acute hemolytic transfusion reaction caused by anti-Sc2. *Transfusion* 2018; **58**:2506–2512
11 Cohen R, Escorcia A, Tasmin F, *et al.*: Feeling the burn: the significant burden of febrile nonhemolytic transfusion reactions. *Transfusion* 2017; **57**:1674–1683
12 Wibaut B, Mannessier L, Horbez C, *et al.*: Anaphylactic reactions associated with anti-Chido Antibody following platelet transfusions. *Vox Sang* 1995; **69**:150–1
13 Lambin P, Le Pennec PY, Hauptmann G, *et al.*: Adverse transfusion reactions associated with a precipitating anti-C4 antibody of anti-Rodgers specificity. *Vox Sang* 1984; **47**:242–249
14 Escorcia A, Tasmin F, Sondi N, *et al.*: The significance of chido/rodgers antibodies in allergic spectrum transfusion reactions; Canadian Society for Transfusion Medicine (CSTM) Annual Meeting. MB, Winnipeg, 2015
15 Pendergrast J, Binnington B, Tong T, *et al.*: Incidence and risk factors for IVIG-mediated hemolysis; American Society of Hematology (ASH) 59th Annual Meeting & Exposition. GA, Atlanta, 2017
16 Yazer MH, Triulzi DJ: The role of the elution in antibody investigations. *Transfusion* 2009; **49**:2395–2399

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Site referrals and investigated reactions
Correlation of final reaction diagnosis with presentation
Overlap of positive serology by testing method
Compliance and Policy Deviation Rates
Rate of serology performance and yield