Received: 1 September 2021 | Revised: 14 December 2021 | Accepted: 3 January 2022

DOI: 10.1002/rth2.12667

rpth research & practice in thrombosis & haemostasis

ORIGINAL ARTICLE

Indeterminate serotonin release assays are associated with a high mortality rate

Shawn Jindal MD[1] | Christopher Leyton MD[1] | Fred Cohen MD[1] | Morayma Reyes Gil MD, PhD[2] | Henny Billett MD, MSc[3]

[1]Department of Medicine, Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, New York, USA

[2]Department of Pathology, Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, New York, USA

[3]Division of Hematology, Departments of Oncology and Medicine, Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, New York, USA

Correspondence
Shawn Jindal, Division of Hematology-Oncology, Montefiore Medical Center, 111 E. 210th St. Bronx, NY 10467, USA.
Email: shjindal@montefiore.org

Funding information
There was no funding source for this study. All authors had access to the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Handling Editor: Dr Suzanne Cannegieter

Abstract

Background: The serotonin release assay (SRA) is considered the gold standard for diagnosis of heparin-induced thrombocytopenia (HIT). Although the SRA holds high sensitivity and specificity when results are definitive, up to 10% of samples from patients with suspected HIT yield "indeterminate" results.

Objectives: We aimed to study the clinical course of patients with indeterminate results.

Methods: We conducted a cohort analysis of 2056 patients that underwent SRA testing.

Results: Of 2056 total patients, 152 (7.4%) had indeterminate assays. The prevalence of thrombocytopenia $<50{,}000 \times 10^6$ was higher in patients with an indeterminate or positive SRA, compared with a negative SRA (39.5% and 40.0% vs. 27.5%, $p < 4.0 \times 10^{-4}$). Patients with an indeterminate SRA were more likely to have been treated in the intensive care unit than patients with a positive SRA (93.3% vs. 73.7%, $p = 0.03$). The mean thrombocytopenia, timing of platelet count fall, thrombosis or other sequelae, and other causes for thrombocytopenia score in patients with indeterminate SRA was 2.9, corresponding to a HIT probability of <5%. Of 152 patients, 128 (78.9%) had heparin-PF4 optical densities (ODs) below 0.60 OD, whereas four patients (2.6%) had ODs above 2.00 OD. Inpatient mortality was significant in patients with indeterminate SRAs compared with positive or negative SRA (49.3% vs. 21.1% and 27.2%, $p < 2.4 \times 10^{-10}$).

Conclusions: Our data suggest that an indeterminate SRA may signal an *in vivo* platelet activation process that is not related to heparin but is associated with increased mortality.

Essentials

- 10% of serotonin release assays to diagnose heparin-induced thrombocytopenia are indeterminate.
- We studied diverse patients with indeterminate serotonin release assays in the Bronx.
- Critically ill patients were more likely to have indeterminate serotonin release assays.
- Patients with indeterminate serotonin release assays had higher mortality.

1 | INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a life-threatening sequela of heparin administration that often results in severe thrombocytopenia and coagulopathy in hospitalized patients. The condition develops in up to 3% of patients treated with unfractionated heparin. Those that develop thrombosis associated with HIT have an estimated 20%–30% mortality.[1]

The serotonin release assay (SRA) is considered the gold standard for diagnosis of HIT, with sensitivity and specificity typically exceeding 95%.[2] The SRA attempts to measure serum-induced serotonin release from platelet granules, which serves as a marker for widespread platelet activation.[3] In the assay, three dilutions of heparin are mixed in separate reactions with patient serum—typically two "low heparin concentration" dilutions and one "high heparin concentration" dilution. In patients with classic HIT, high levels of serotonin release are typically observed in the "low heparin concentration" reactions, whereas low serotonin release is typically observed in the "high heparin concentration" reaction because of saturation of heparin-binding sites on PF4 molecules.[4]

"Indeterminate" serotonin release assay results occur when elevated serotonin release is present at both low and high concentrations of heparin. Studies have approximated the prevalence of an indeterminate SRA to range between 4% and 10%.[5] The implications of indeterminate serotonin release assays have not been widely studied, and little is known about what causes serotonin release to be elevated in all three heparin dilutions in the assay. Hypotheses include the presence of heparin-binding proteins that interfere with the assay, high-titer HLA class I alloantibodies, or immune complexes (either present *in vivo*, or generated *ex vivo* when the heat inactivation step—used to inactivate residual thrombin—results in formation of immune complexes).[4] The clinical course of patients with indeterminate SRA results is also poorly documented. Available data suggest that when patients with a history of SRA-confirmed HIT are reexposed to heparin, 4%–5% experienced heparin-associated complications.[6] No study, however, has evaluated heparin reexposure in patients with previously indeterminate SRAs.

We conducted a cohort analysis of patients that underwent SRA testing to evaluate etiologies, platelet trends, physician response, and patient outcomes in this SRA indeterminate group compared with those testing as positive and negative.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

We performed a single-center cohort analysis of data on all adult patients (aged ≥18 years) at Montefiore Medical Center who underwent SRA testing between January 1, 2014, and December 31, 2018, as part of the evaluation for HIT.

2.2 | Data collection

Data were obtained from the electronic medical record and Clinical Looking Glass, an interactive database software application developed at Montefiore Medical Center that integrates demographic, clinical, and administrative datasets for the purpose of clinical research. SRA results were considered negative by laboratory algorithm. Serotonin release results <19% at all heparin dilutions (0.1, 0.5, and 10 U/ml) are considered negative; SRA results ≥19% in the 0.1 and 0.5 U/ml dilution reactions and <19% in the 10 U/ml reaction are considered positive. SRA results are considered indeterminate when serotonin release exceeds 19% in all three dilution reactions. In addition to electronic medical record and Clinical Looking Glass data extraction, charts of patients with positive and indeterminate SRA results were reviewed in detail to obtain variables including patient demographics, type of heparin product received before SRA testing, associated diagnoses, timing of heparin exposure, heparin-PF4 optical densities (ODs), platelet trends, incidence of clot, clinical course, frequency of heparin reexposure, and mortality.

2.3 | Statistical analysis

Statistical analysis was performed using means and standard deviations and median and interquartile ranges as appropriate for normal or non-normal distributions. Comparisons were made using chi-square, ANOVA, or *t* tests. If sample sizes were small (<5), Fisher exact test was used. Significance was assumed at an alpha ≤ 0.05.

3 | RESULTS

We identified 2056 adult patients who underwent SRA testing between 2014 and 2019 as part of evaluation for HIT. Of these, 90 patients (4.4%) had positive SRA results, 1814 patients (88.2%) had negative SRA results and 152 patients (7.4%) had indeterminate SRA results. Patients with indeterminate SRA were significantly younger than patients with positive or negative SRA results (Table 1). Patients who had negative serotonin release assays were also less likely to have significant thrombocytopenia. Of the 1814 patients that tested negative, 498 (27.5%) had platelet counts below 50×10^9/L at the time of SRA testing, compared with 40.0% and 39.5% in the positive SRA and indeterminate SRA groups, respectively ($p < 4.0 \times 10^{-4}$, Table 1).

In the vast majority (94.1%) of cases, patients were tested for anti-PF4/heparin antibodies concurrently with their SRA testing (Table 2). Of the 152 patients with indeterminate SRAs, 120 (78.9%) had PF4 ODs below 0.60, indicating a low likelihood of true HIT. In 19 cases (12.5%), PF4 ODs were in an indeterminate range of 0.60–2.00. In four cases (2.6%), PF4 ODs exceeded 2.00 (Table 2).

Among the 152 patients with indeterminate SRA results, the mean thrombocytopenia, timing of platelet count fall, thrombosis

TABLE 1 Demographics of 2056 patients with SRA testing results

	Positive SRA $N = 90$	Negative SRA $N = 1814$	Indeterminate SRA $N = 152$	p^*
Age, y (mean ±SD)	65.6 ± 17.7	63.8 ± 17.2	57.5 ± 17.7	$<1.0 \times 10^{-4}$
Female, *n* (%)	47 (52.2)	869 (47.9)	87 (57.2)	0.7
Hispanic ethnicity, *n* (%)	26 (28.9)	527 (29.1)	50 (32.9)	0.6
Race				
Black, *n* (%)	27 (30.0)	599 (33.0)	40 (26.3)	$<1.0 \times 10^{-4}$
White, *n* (%)	24 (26.7)	394 (21.7)	32 (21.1)	
Asian, *n* (%)	3 (3.3)	23 (1.3)	3 (2.0)	
Unknown/ refused, *n* (%)	10 (11.1)	27 (14.9)	29 (17.8)	
Platelet count $<50 \times 10^9$/L, *n* (%)	36 (40.0)	498 (27.5)	60 (39.5)	$<4.0 \times 10^{-4}$
Died, *n* (%)	19 (21.1)	493 (27.2)	75 (49.3)	$<2.4 \times 10^{-10}$

Abbreviations: SD, standard deviation; SRA, serotonin release assay.

*Probability of observed differences between the positive SRA, negative SRA, and indeterminate SRA cohorts.

TABLE 2 Clinical course of the 152 patients with indeterminate SRA according to PF4 Result

PF4 Result	OD <0.60	OD 0.60–2.00	OD > 2.00	ELISA untested
n (%)	120 (78.9)	19 (12.5)	4 (2.6)	9 (5.9)
4Ts score, mean (SD)	2.89 (1.2)	2.73 (1.0)	2.66 (1.7)	2.99 (1.4)
Heparin continued or reexposed, *n* (%)	62 (51.7)	9 (47.4)	2 (50.0)	5 (55.6)
Platelet drop, *n* (%)	4 (6.5)	0 (0)	2 (100)	1 (20)
Acute VTE, *n* (%)	3 (4.8)	0 (0)	1 (11.1)	0 (0)
Died during admission, *n* (%)	58 (48.3)	10 (52.6)	1 (25.0)	6 (66.7)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; OD, optical density; SD, standard deviation; SRA, serotonin release assay; VTE, venous thromboembolism.

TABLE 3 Causes of death in patients with indeterminate and positive SRAs

	SRA indeterminate ($n = 152$)	SRA positive ($n = 90$)	p
Mortality, *n* (%)	75 (49.3)	19 (21.1)	$<1.0 \times 10^{-5}$
Treated in intensive care unit, *n* (%)	70 (93.3)	14 (73.7)	0.03
Cause of death, *n* (%)			
Sepsis	33 (44.0)	6 (31.6)	0.33
Respiratory failure	14 (18.7)	5 (26.3)	
Cardiogenic shock	15 (20.0)	2 (10.5)	
Thromboembolism	1 (1.3)	0 (0.0)	
Hemorrhagic shock	5 (6.7)	1 (5.3)	
Stroke	2 (2.7)	2 (10.5)	
Other/data insufficient	5 (6.7)	3 (15.8)	

Abbreviation: SRA, serotonin release assay.

or other sequelae, and other causes for thrombocytopenia (4Ts) score was just 2.9, corresponding to a HIT probability of <5%. Interestingly, the 4Ts score was not very different across the spectrum of OD groups. The decision to restart heparin did not appear to be dependent on the PF4-heparin antibody result or the 4Ts score. Of the 152 indeterminate SRA patients, 78 (51.3%)

were either continued or were reexposed to heparin; in 71 of these 78 cases (91.0%), the platelet count stabilized or improved despite heparin exposure. In the remaining seven (9.0%) cases, the thrombocytopenia did not stabilize or improve but heparin was continued, no alternative HIT therapy was given, and no acute thrombotic events followed. Four of the 71 patients (5.6%) that continued or were reexposed to heparin were noted to have an acute venous thromboembolism (VTE; Table 2). Of note, three of these occurred in the patients with normal PF4 ODs and the fourth had an OD of 2.11.

Patients with a positive SRA or a negative SRA had similar mortality rates (21.1% vs. 27.2%, respectively), but patients with an indeterminate SRA had a significantly higher mortality rate of 49.3% (Table 1, $p < 2.4 \times 10^{-10}$). Many of the patients with an indeterminate SRA were critically ill at the time HIT was suspected, and their deaths were from many causes including, but not limited to, sepsis, cardiogenic shock, and hypoxic respiratory failure. Patients with an indeterminate SRA were more likely to have been treated in the intensive care unit than patients with a positive SRA (93.3% vs. 73.7%, $p = 0.03$, Table 3).

4 | DISCUSSION

Given the potential severity of HIT, the question of "HIT or not HIT?" must be addressed expeditiously.[7] When provided with an indeterminate SRA result, clinicians are often left with uncertainty on how to proceed in managing thrombocytopenic patients being given heparin. Currently, few data exist in the literature to provide clarity on the clinical course of patients with indeterminate SRA.

Guideline-directed practice when evaluating patients for HIT includes tabulating a 4Ts score.[8] We found that most patients who were tested did not have significant thrombocytopenia, although we did not chart the time course of the decreasing platelets for each patient. We did find, however, that, patients with negative SRA had platelet counts above 50×10^9/L at the time of SRA testing more frequently than patients with positive SRA or indeterminate SRA. Interestingly, the degree of thrombocytopenia in patients with positive SRA results and indeterminate SRA results was very similar. Patients with true HIT are known to develop severe thrombocytopenia, frequently falling below counts of 50×10^9/L. Our data suggest that platelet counts in this positive SRA cohort are similar to the counts in the indeterminate SRA cohort, despite a low likelihood that the latter includes patients with true HIT.[9]

To explore the likelihood that patients with indeterminate SRA truly had HIT, we evaluated these patients' 4Ts scores before HIT evaluation, their anti-PF4/heparin antibody ODs, and their clinical course, specifically if heparin was never discontinued or if they were reexposed to heparin during their hospital stay. The majority of patients had anti-PF4 ODs below 0.60, indicating a low likelihood of true HIT. Very few patients had ODs exceeding 2.0, which is often associated with a higher likelihood of true HIT.[9] Approximately one-half of patients in our study who received indeterminate SRA results were either continued on heparin after their laboratory results or they were reexposed to heparin later on in the admission; 91% of these patients did not exhibit further platelet drop upon continuation/reexposure, with platelet counts typically recovering despite heparin administration. Additionally, few acute VTEs were seen in these patients upon heparin continuation/reexposure. In combination with very low mean 4Ts scores, it appears unlikely that these patients with indeterminate SRA truly had HIT.

Cases that were most challenging to assess were the two in which patients had OD > 2.00, highly suggestive of true HIT, along with either acute VTE (one patient) or platelet drop following continued heparin exposure (two patients). One patient had all three traits that were highly suggestive of true HIT: OD > 2.00, acute VTE, and continued platelet drop while on heparin. Despite the indeterminate SRA of this patient, it is certainly possible that this was a true case of HIT. Despite its high sensitivity, there are limitations with the SRA that may lead to true HIT cases being deemed indeterminate, which may have been the case here. The pattern of serotonin release in the SRAs of each case were also closely examined. In both cases, serotonin release exceeded 90% in all three heparin dilutions, consistent with an indeterminate SRA result.[10]

The association of higher mortality rates in patients with indeterminate SRA results, compared with both positive and negative SRA patients, is interesting and bears further investigation. The data suggest that patients with true HIT, as suggested by positive SRA results, had lower mortality than other patients. A possible explanation for this is that patients with other illnesses serious enough to cause significant thrombocytopenia have worse outcomes than patients with drug-related thrombocytopenia. Particularly for those patients with indeterminate SRA and an almost 50% mortality, it may be that illnesses causing thrombocytopenia and immune complex and/or cytokine-mediated platelet activation, as evidenced by the serotonin release, are particularly severe. In this sense, the SRA may be serving as a surrogate biomarker in the same sense as the D-dimer. Thus, although we know HIT to be a potentially fatal condition with substantial risk for thrombosis, the indeterminate SRA results may point toward an even graver state of advanced illness. The higher prevalence of intensive care unit admission in the cohort of patients with indeterminate SRA compared with patients with positive SRA provides some support for this hypothesis.

5 | CONCLUSION

Our study shows that most patients who receive an indeterminate SRA result do not have true HIT but that this group is associated with a poorer prognosis compared with patients with positive SRA or negative SRA. This conclusion may be helpful to clinicians who are currently uncertain on how to proceed with management of patients with significant thrombocytopenia who have indeterminate SRA results. These patients may not require nonheparin alternative

therapeutic anticoagulation but may presage a heightened response to a life-threatening disease for which the therapies should be geared to the primary illness. We believe further studies should be conducted that explore the mechanism behind indeterminate SRA results. Further research will help to better understand the circumstances that lead to an indeterminate SRA result, leading to a deeper understanding of the assay, its utility, and its reliability in different clinical contexts.

RELATIONSHIP DISCLOSURE

All authors have no competing financial interests to declare.

AUTHOR CONTRIBUTIONS

Shawn Jindal undertook conception, data collection, statistical analysis, and manuscript writing. Christopher Leyton undertook conception and data collection. Fred Cohen undertook conception. Morayma Reyes Gil wrote the manuscript. Henry Billett provided conception and manuscript writing.

REFERENCES

1. Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med*. 1995;332(20):1330-1335. doi:10.1056/NEJM199505183322003
2. Warkentin TE, Arnold DM, Nazi I, Kelton JG. The platelet serotonin-release assay. *Am J Hematol*. 2015;90(6):564-572. doi:10.1002/ajh.24006
3. Vayne C, Guéry EA, Charuel N, et al. Evaluation of functional assays for the diagnosis of heparin induced thrombocytopenia using 5B9, a monoclonal IgG that mimics human antibodies. *J Thromb Haemost*. 2020;18(4):968-975. doi:10.1111/jth.14749
4. Warkentin TE, Sheppard JI, Smith JW, et al. Combination of two complementary automated rapid assays for diagnosis of heparin-induced thrombocytopenia (HIT). *J Thromb Haemost*. 2020;18(6):1435-1446. doi:10.1111/jth.14794
5. Moore JC, Arnold DM, Warkentin TE, Warkentin AE, Kelton JG. An algorithm for resolving 'indeterminate' test results in the platelet serotonin release assay for investigation of heparin-induced thrombocytopenia. *J Thromb Haemost*. 2008;6(9):1595-1597. doi:10.1111/j.1538-7836.2008.03047.x
6. Dhakal P, Giri S, Pathak R, Bhatt VR. Heparin reexposure in patients with a history of heparin-induced thrombocytopenia. *Clin Appl Thromb Hemost*. 2015;21(7):626-631. doi:10.1177/1076029615578167
7. Zwicker JI, Uhl L, Huang WY, Shaz BH, Bauer KA. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J Thromb Haemost*. 2004;2(12):2133-2137. doi:10.1111/j.1538-7836.2004.01039.x
8. Arepally GM, Ortel TL. Clinical practice. Heparin-induced thrombocytopenia. *N Engl J Med*. 2006;355(8):809-817. doi:10.1056/NEJMcp052967
9. Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost*. 2008;6(8):1304-1312. doi:10.1111/j.1538-7836.2008.03025.x
10. Zheng G, Streiff MB, Takemoto CM, et al. The clinical utility of the heparin neutralization assay in the diagnosis of heparin-induced thrombocytopenia. *Clin Appl Thromb Hemost*. 2018;24(5):749-754. doi:10.1177/1076029617721013

How to cite this article: Jindal S, Leyton C, Cohen F, Reyes Gil M, Billett H. Indeterminate serotonin release assays are associated with a high mortality rate. *Res Pract Thromb Haemost*. 2022;6:e12667. doi:10.1002/rth2.12667