Platelet Aggregation Quality Control in a Multicenter, Phase 2 Clinical Trial

Platelet aggregation assays provide vital information on pharmacodynamics and population variability in clinical trials of antiplatelet agents. However, nonexpert operators in multiple trial sites may generate artifactual results, reducing data quality.

This study aimed to determine whether platelet aggregation assays performed in a phase 2 trial of a novel antiplatelet agent were of sufficient quality to assess the drug's effectiveness. A specialist contract research organization with expertise in platelet function analysis developed procedures for training and providing feedback to operators, and a data monitoring and review process, classifying data as usable or artifactual.

Usable platelet aggregation data was obtained from over 90% of assayed time points. Site choice, training, and ongoing support contributed to the generation of high-quality aggregation data, with the assay success rate improving during the trial. Independent data review objectively identified artifactual platelet aggregation assay results, allowing better use of study data to determine drug effects and population differences.

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INTRODUCTION

Platelet activation is central to arterial thrombosis, the world's leading cause of death (1). Platelet function and the effect of most antiplatelet drugs may be assessed using the platelet aggregation assay, which measures the light transmission of a stirred suspension of platelets (2). Optical measurement of platelet aggregation was first described nearly 50 years ago (3,4), and is used in research centers investigating platelet function (5). Aggregation in ex vivo samples reflects the platelet function status of a subject, including the effects of disease, drug regimens, and individual variation in response.

Inhibition of aggregation may be the only indication of clinical effectiveness of antiplatelet agents (6) before large phase 3 efficacy trials, and is required for novel antiplatelet drugs by the European Medicines Agency (EMA, formerly EMEA) (7). While aggregation correlates well with plasma drug levels for drugs with a reversible effect (8), many antiplatelet drugs are essentially irreversible inhibitors of their target (clopidogrel, PAR1 antagonists, aspirin), in which case plasma drug levels are uninformative (9) and a functional assay such as aggregation is the only way to detect the drug's effect.

Platelet aggregation also captures population response data such as failed drug response (so-called aspirin and clopidogrel resistance) (10). Since it measures the overall platelet function, aggregation is suited to detecting such heterogeneous responses, which may be due to variation in the population of drug metabolism or effectiveness.

While useful, platelet aggregation is a novel procedure for many clinical trial sites. Inexperienced operators require training in steps such as blood sample processing, instrument setup and calibration, sample analysis, and data interpretation. Essentially, a specialized research procedure will be performed by nonexperts to generate clinical trial data. There may be concerns about data quality and it is prudent to apply data review processes.

Other assays in clinical trials are quality controlled by being centrally performed (such as drug or biomarker measurement) or subjected to expert scrutiny (such as ECG). In contrast, there are no established procedures to identify artifactual or out-of-range assay results with platelet aggregation, which may lead to erroneous conclusions. For instance, a sample may be incorrectly processed, and no aggregation seen, suggesting complete platelet inhibition, where the actual platelet function is unaffected. In a

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small phase 2 trial, a few such errors could lead to inappropriate decisions regarding drug effectiveness. Review of the aggregations, independent of the study sites and sponsor, allows identification of such artifacts and appropriate analysis of the drug's effects.

This study aimed to assess the ability of heterogeneous sites to perform platelet aggregation assays in a phase 2 clinical trial and to determine the validity of the resultant data. In a study of a novel antiplatelet agent, an independent specialist contract research organization (CRO) with extensive experience in the area developed a training program for operators and reviewed all the platelet aggregation data generated. The majority of data were generated at sites and by personnel naive of platelet aggregation. Certain errors in performing the assay were relatively common, although these were reduced through training and feedback. Overall data quality was satisfactory, with over 90% of assayed time points providing good-quality platelet aggregation data.

MATERIALS AND METHODS

SETTING

The aggregation assays were performed during phase 2 double-blinded trials investigating a novel antiplatelet agent in two populations: coronary artery disease (CAD, patients with stable disease) and acute coronary syndrome (ACS). Platelet function assessment was a secondary objective, performed at various time points over the course of the trials in intensive substudies at sites in the United States, Canada, Argentina, South Africa, the UK, Italy, Hungary, Poland, Romania, Russia, India, and Australia. Each substudy aimed to recruit 80 patients randomized to placebo, or one of three different daily doses of study drug. Studies were conducted in accordance with the principles of the Declaration of Helsinki, ICH GCP, and relevant national regulatory requirements.

AGGREGATION ASSAYS

Platelet aggregations were measured using PAP-8E aggregometers (Bio/Data Corp, Horsham, PA), using essentially standard procedures. Customized software facilitated compliance with the trial protocols. Blood was drawn into citrated 3.5 ml Vacutainers. Platelet-rich plasma (PRP) and platelet poor-plasma (PPP) were generated by short, high-speed spins, using a custom centrifuge (Bio/Data) and aspirated. Single-day aliquots of platelet agonists and reconstitution reagents were supplied. In the CAD study, an agonist (agonist 1) expected to be inhibited by study drug was used to induce aggregation. In the ACS study, a control agonist (agonist 2) likely to be unaffected by the study drug was also used. Each agonist was assayed in duplicate. Aggregation assays were of 6 minutes' duration, with data recorded electronically. Data at each site were periodically copied onto CD and sent to the platelet specialist CRO, who performed independent data review (Java Clinical Research, Dublin, Ireland).

Platelet aggregation is measured as a percentage, with 0% corresponding to the optical density of the relatively opaque, dense suspension of PRP. One hundred percent aggregation is equivalent to the relatively transparent PPP (1) (Figure 1). These reference points are set for each sample because of variation in factors such as platelet count and plasma opacity. Thus, blood is centrifuged to generate PRP over a hematocrit layer. This is aspirated and the remaining blood further centrifuged to generate PPP. A tube of PPP sets the 100% aggregation reference point. This is replaced by the assay tube containing PRP and a magnetic stir bar. When the assay proper is started, the initial light transmission of PRP sets the 0% aggregation level. Platelet activators cause aggregation, reducing the number of separate particles, and hence opacity, of the sample. Percentage aggregation is recorded to give a graph (tracing) of platelet aggregation over time. The principles of the assay are outlined in Figure 1. The assay was performed in 225 µl PRP, with 25 µl agonists. The dilution effect of agonist addition is corrected by re-zeroing the aggregation percentage as reagent is added. Factors affecting the setting of the calibration points, altering light

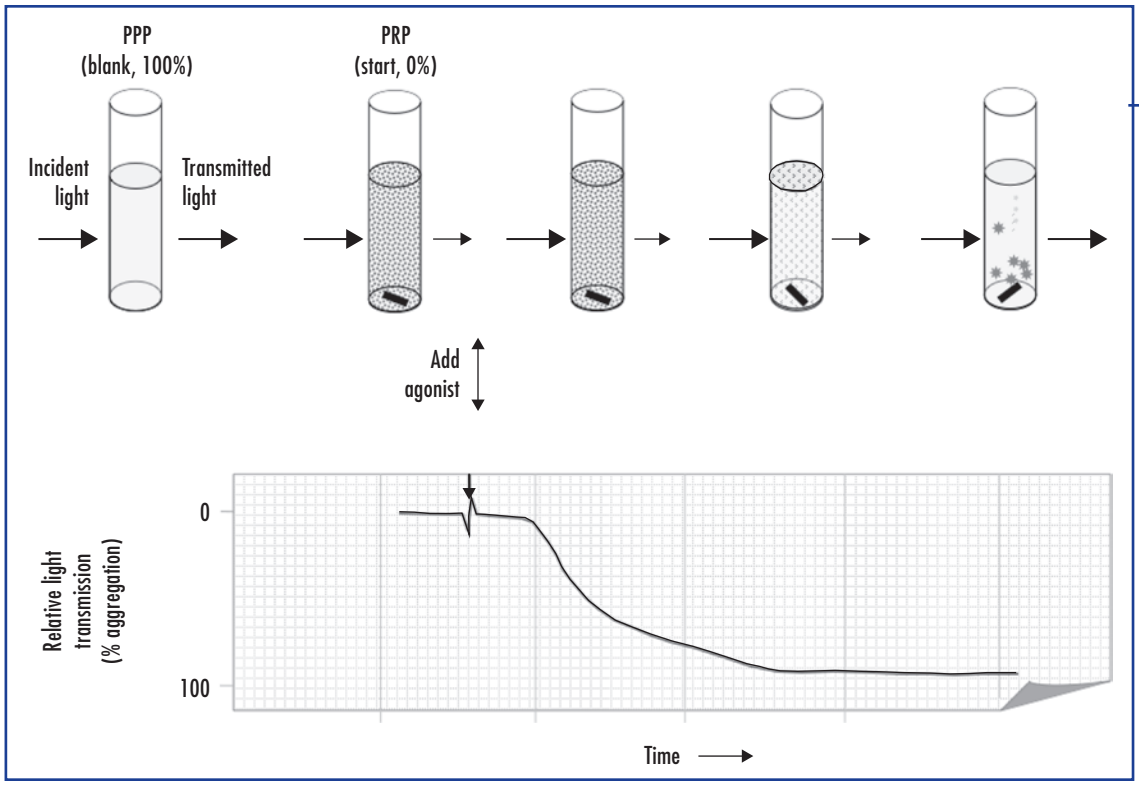


FIGURE 1

Platelet aggregation assay. Platelet aggregation is measured as the change in light transmission of a platelet-rich plasma sample challenged with agonist or other reagent. The light transmission is calibrated between reference values of 100% (determined by blanking with the patient's PPP) and 0% (determined at the start of the assay from PRP). As aggregation progresses, platelets cohere into a small number of aggregates, allowing more light to pass through the sample.

transmission during the assay, or otherwise affecting the assay may cause artifactual results.

OPERATOR TRAINING

Site Setup. One trained operator with no other role in the trial (to prevent unblinding) was required at each substudy site. Site selection usually, but not in every case, identified sites that were appropriately committed to the substudy.

Operator Training. Platelet aggregation operators were instructed by trainers from the equipment manufacturers (Bio/Data Corp), the independent data reviewers (Java Clinical Research), or both. This included blood sample processing, reagent reconstitution, performing the assay, using software, and data transfer. Acquisition of blood samples required for training was left to the discretion of local medical staff at each site, as is usual in such situations. Following training, prospective operators were required to repeat the entire procedure independently and submit these aggregation tracings to the independent reviewer for validation. If satisfactory, the operators were validated to run trial assays. If unsatisfactory, advice on assay technique was given and operators repeated the validation procedure.

Ongoing training initiated by the independent reviewers included the following:

- Operators were free to practice the procedure and submit tracings for review and advice.
- A training video demonstrating the procedure was produced and distributed.
- A monthly newsletter illustrating common errors and corrective actions was circulated.
- Webcasts to sites highlighted quality issues.
- Sites with a delay from operator training to first patient recruitment were contacted to ensure operator familiarity with the aggregation procedure.

DATA REVIEW

Data sent from sites was reviewed by independent data reviewers in the platelet specialist CRO for data quality. To assist in the identification of errors, the independent reviewers simu-

TABLE 1

Errors Encountered in Platelet Aggregation Assays

Error	Code	Cause	Manifestation	Figure
Incorrectly prepared PPP	1	Carelessly aspirated PPP containing platelets or WBCs. Results in 100% mark for aggregation being incorrectly set.	Increased apparent aggregation, often >>100%.	3A, 3B
Incorrect tube placement	2	Tube of PRP not pushed fully down to the bottom of the assay well.	Subsequent movement of test tube results in a jump in light transmission.	3C, 3D
Excessive noise	3	Poor pipetting or insufficient sample may result in bubbles in the PRP.	Random fluctuations in the light transmission and apparent aggregation.	3E, 3F
Agonist addition error	4	Omitting agonist, or not adding it directly to PRP, or re-zeroing the tracing inappropriately.	No aggregation or delayed initiation of aggregation or artifactual curve shape.	3G, 3H
Hemolysis	5	Contamination of PRP, PPP, or both with red blood cells due to sample hemolysis or careless preparation of samples.	Suppressed aggregation curves due to similarity in optical density between PPP and PRP.	3I, 3J
Operator judgment	6	Operator indicates the aggregation is unsatisfactory. May be due to one of the issues in this table, or others (eg, lipemic sample).	May be explicit (operator notes issue in a comment) or implicit (operator repeats aggregations due to problem).	Not shown
Suspected concomitant medications	7	Glycoprotein IIb/IIIa antagonists (used for acute prevention of thrombosis during coronary interventions) abolish platelet aggregation in response to any stimulus.	Simultaneous inhibition of responses to agonist 1 and agonist 2 in early time points of patients enrolled in the ACS study.	3K, 3L
Baseline activation of platelets	8	Platelets activated before start of assay by poor phlebotomy technique, ambient temperatures, or prolonged period ex vivo.	Stirring alone, without the addition of agonists, results in aggregation or change in optical density.	3M, 3N
Other reasons	9	Various other causes may result in unusable data. Where an assay is repeated unnecessarily (the original attempt produced usable data).	Various.	Not shown

lated likely errors in performing the assay, including mistakes in sample preparation, reagent reconstitution, and assay performance, together with issues with blood samples rendering the assay impossible to perform.

Based on these simulations, aggregations from sites were assigned a code describing their status. Code 0 signified usable data with no major quality issues, while codes from 1 to 8 indicated the most likely cause of data being unusable, where this could be determined with reasonable confidence. A code of 9 indicated other unusable data, including where the cause could not be determined (Table 1).

All data were reviewed and coded by the same individual on an ongoing basis. Where avoidable errors were noted, operators were informed of the likely cause of the error and instructed how to avoid repeating the error. To ensure the objectivity of the review process, some aggrega-

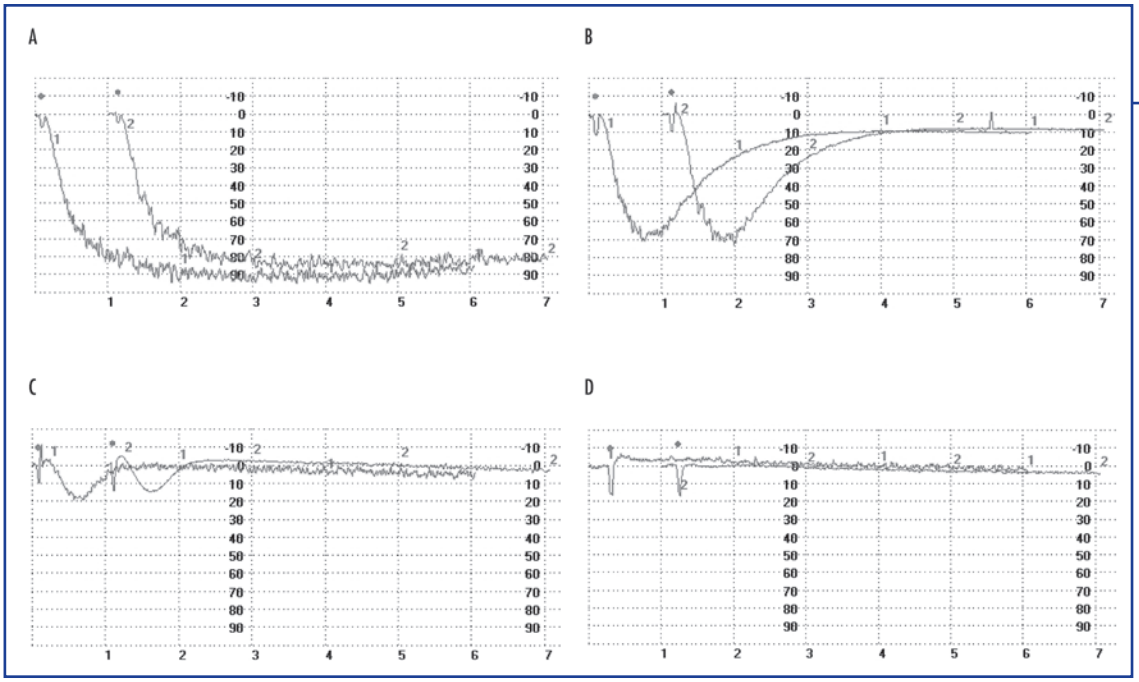


FIGURE 2

Typical aggregation responses. A: Typical response generated by agonist 1 in patients before the administration of study drug. B–D: Various degrees of inhibition of aggregation responses to agonist 1, consistent with the presence of various concentrations of study drug.

tions were reviewed and coded by a second independent reviewer. These were compared with the codes from the first reviewer, and any disparate codes reconciled by agreement between the reviewers.

RESULTS

TYPICAL AGGREGATION RESPONSES

Platelet responses to agonist 1 varied from a rapid and full aggregation to complete inhibition, almost certainly due to varying plasma concentrations of study drug. Partially inhibited responses typically showed transient aggregation that reverted to baseline. Examples from study subjects are shown in Figure 2, A–D. In the ACS study, responses to agonist 2 were unaffected by study drug and varied based on other factors (not shown).

UNUSABLE DATA

In addition to these valid responses, aggregation tracings with clear errors were received from trial sites. These were classified based on simulations performed by the independent reviewers. The causes and results of such errors are listed in Table 1 and shown in Figure 3, with simulations (A, C, E, G, I, K, M) and errors from sites (B, D, F, H, J, L, N) shown for comparison.

USABLE DATA

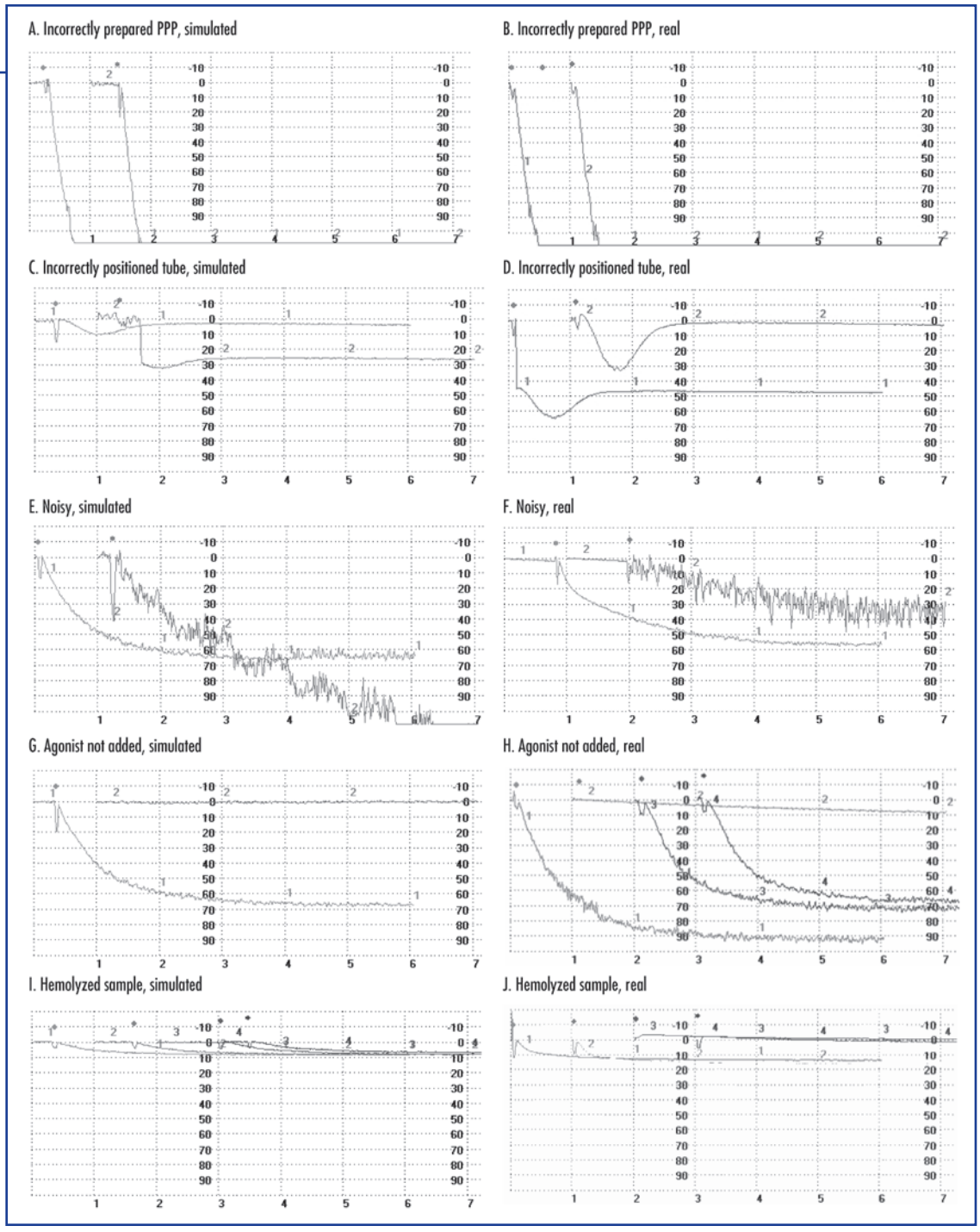
In total, 83.6% of scheduled platelet aggregation assays were performed, with some optional time points omitted, and others missed due to reasons such as patient nonattendance.

Overall, 5,906 platelet aggregations were reviewed, of which 369 were redundant (repeated attempts of the same time point). Henceforth, only nonredundant assays are considered. Of these, 87.2% were usable, with 83.6% of time points having two usable tracings, and 90.6% at least one usable duplicate. All data, including redundant data, were transferred to the sponsor's data management team with the independent data reviewers' assessment of data usability.

CLASSIFICATION OF DATA

The most common cause of unusable data was incorrectly prepared PPP (code 1, 3.9% of assays), followed by interfering concomitant medication (code 7, 1.8%). Other errors had an incidence of less than 1.5% (Figure 4). Errors were unevenly distributed across sites, with, for ex-

FIGURE 3



Simulated and real errors. Artifactual results in platelet aggregation data were categorized by likely cause of error, based on simulated assay errors performed by the independent data reviewers. A, C, E, G, I, K, M: Simulated errors. B, D, F, H, J, L, N: Examples of errors seen in trial data. A, B: Contaminated PPP results in an incorrect calibration of the 100% mark, producing an exaggerated aggregation tracing. C, D: An incorrectly placed test tube moves during the assay, giving a jump in the light transmission and apparent platelet aggregation (C, tracing 2; D, tracing 1). E, F: Bubbles or insufficient PRP results in random fluctuations in light transmission (E, tracing 2; F, tracing 2). G, H: Omission of agonist results in no aggregation (note no downward deflection [dilution effect] in G tracing 2 and H tracing 2). I, J: Hemolysis results in suppressed tracings, simulated by the addition of red blood cells to samples. Other parameters (such as high absolute optical density) distinguish this from concomitant medications.

ample, some showing more frequent errors due to preparing PPP, while interfering concomitant medication was only seen at some sites in the ACS study where GP IIb/IIIa antagonists were commonly used.

Data codes 1–4 reflect errors in performing the assay. In most of these cases, the operator could have generated usable data if the assay had been correctly performed. These errors caused the loss of 6.6% of data. In contrast, codes 5–8 (5.0% of data) largely reflect issues rendering the sample impossible to analyze, such as hemolysis or other drug effects (Figure 4).

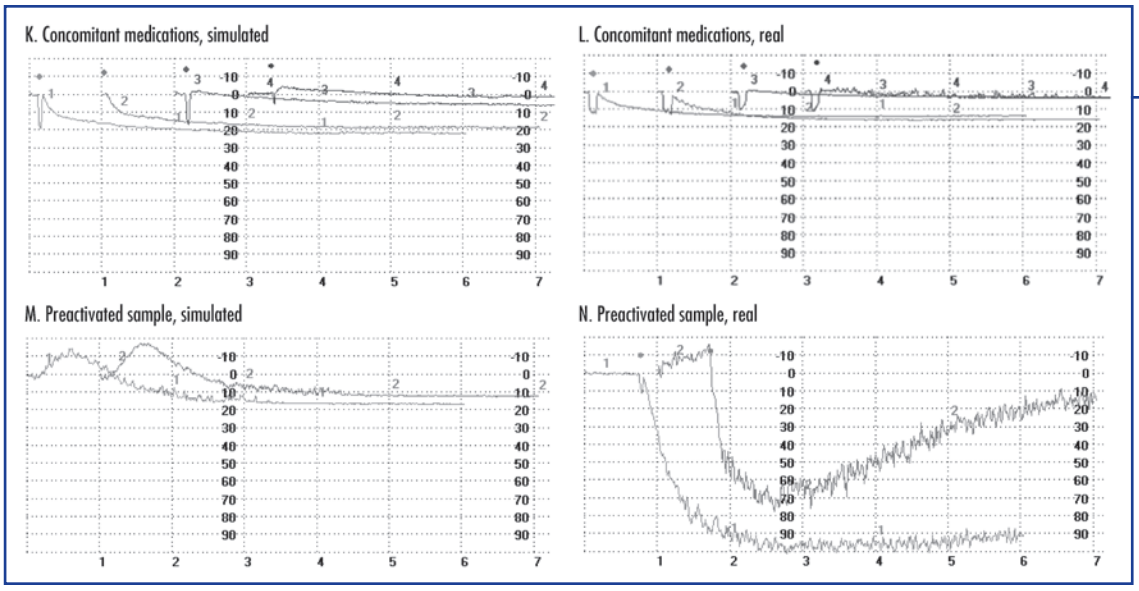


FIGURE 3

(*Continued*)
K, L: Concomitant medications. GP IIb/IIIa antagonists prevent platelet aggregation from any stimulus. Such inhibition does not reflect the action of study drug. Note that responses to agonist 1 and agonist 2 are both strongly suppressed. Operator comments and the timing of such responses helped distinguish this from hemolysis. M, N: Activated sample. Poor sample handling results in platelets that change optical density upon stirring in the absence of any agonist (M, tracing 1 and 2) or before agonist addition (N, tracing 2).

DATA QUALITY

Data were monitored by the independent reviewers as these trials progressed. Operators were informed of avoidable errors and appropriate corrective action. Data quality improved as the trial progressed. Comparing the first quintile of assays performed in each site to the last, usable data increased from 79% to 89%. Anecdotally, some operators indicated that based on feedback from the data reviewers, they

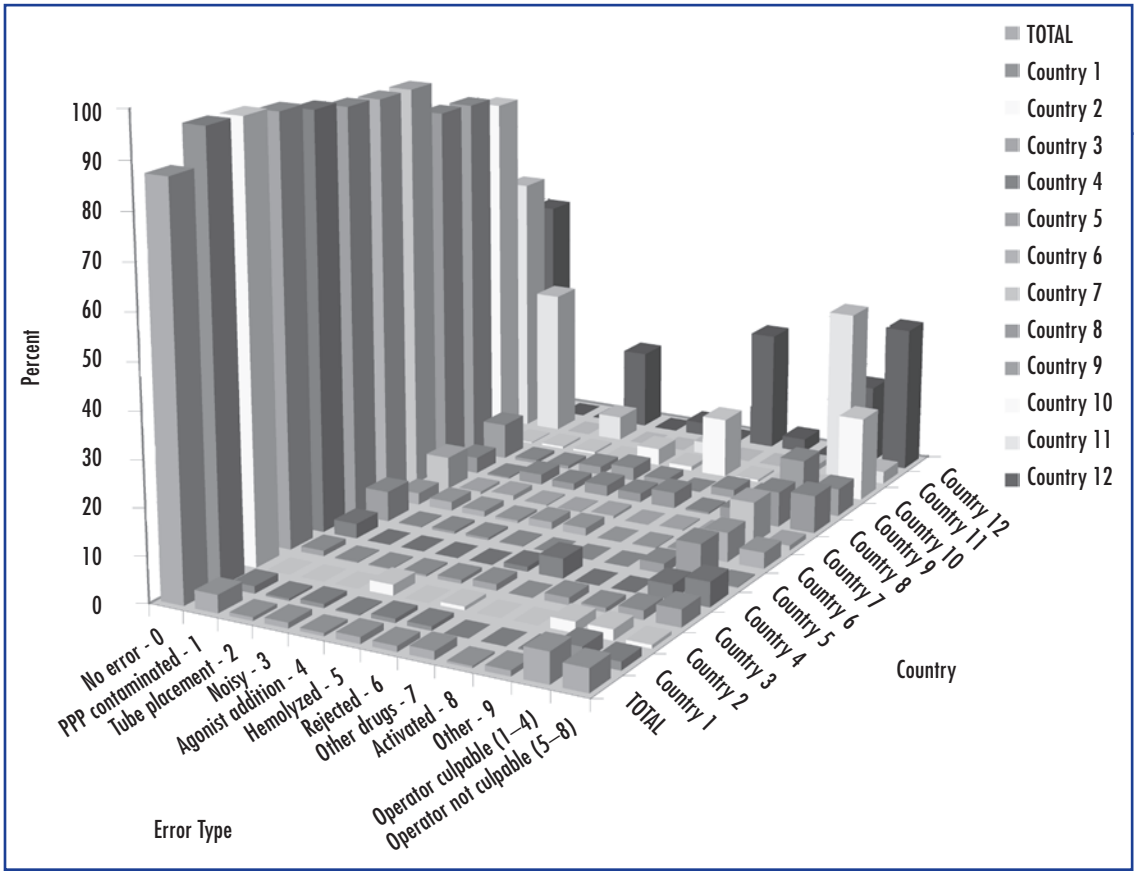
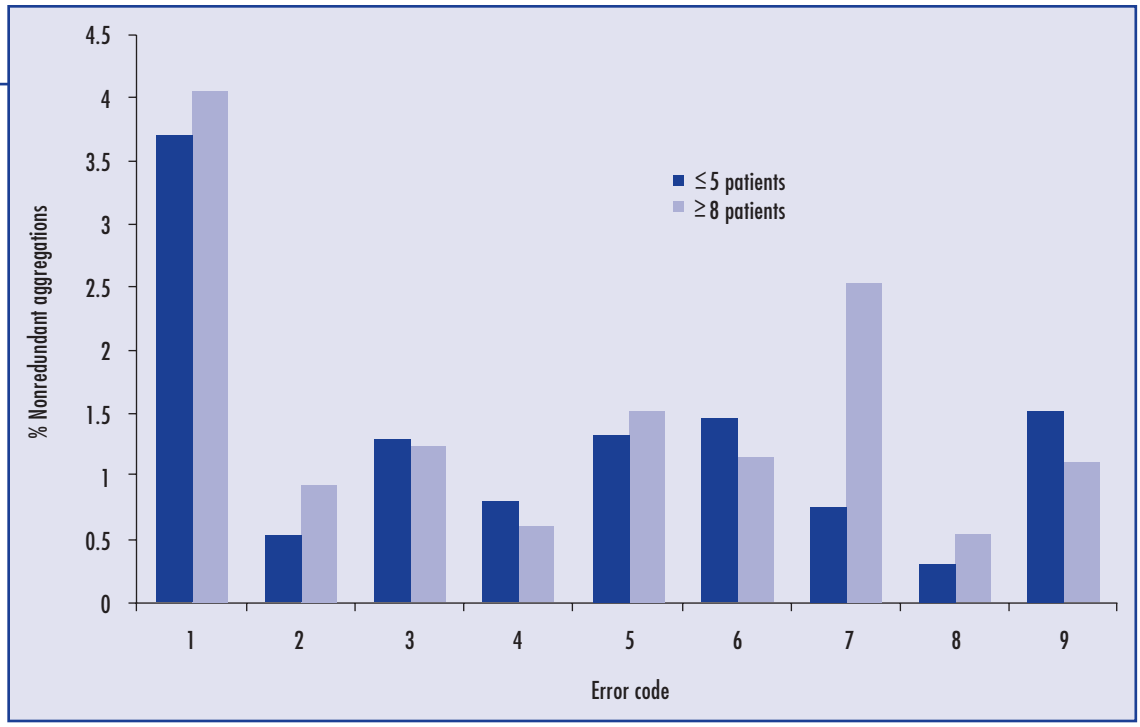


FIGURE 4

Variation in error rates by category and country. Usability codes were assigned as detailed in the text and Table 1 and illustrated in Figure 3. Also shown are the summed rates due to mistakes in performing the assay (codes 1–4) and those for which the operator may be blameless (codes 5–8).

FIGURE 5



Types of error in high and low recruiting sites. There was little relationship between the rate of patient recruitment in a site and the rates or types of error seen. Two high-recruiting sites where GP IIb/IIIa inhibitors were frequently used contributed the bulk of aggregations with error code 7 (concomitant medications).

recognized and repeated unsatisfactory tests to give usable data.

Recruitment rate did not appear to be a factor in determining assay performance. Of 36 active sites, just eight recruited over 60% of substudy patients. While sites with five or fewer patients had 88.3% usable data, those with eight or more patients had 86.3% usable data, and the types of error seen were similar in sites with high and low recruitment rates ($P = 0.67$ by single factor ANOVA for variation between these site categories in the type of error seen, Figure 5).

COMPARISON OF TWO INDEPENDENT REVIEWERS

A subset of data was reviewed by a second independent reviewer. Data reviewed a second time included all data coded as unusable by the first reviewer, a sample (10%) of data coded as usable, and any associated tracings. Comparison of the two reviews revealed a small number of discrepancies, most often due to ambiguous errors. The second review resulted in a change in the usability status in less than 0.5% of aggregations.

DISCUSSION

Platelet aggregation is a critical assay in the clinical development of antiplatelet therapies, providing pharmacodynamic data and assessing population responses. Other assays are available, but the standard remains light-transmission aggregometry tailored to the particular pathway of interest. This study demonstrates that with appropriate training and support, inexperienced operators can perform platelet aggregation assays to an acceptable standard in a clinical trial setting. Independent review ensures that data suitable for analysis are identified for the study sponsor. Poor preparation of PPP was the most common source of error. Other factors resulting in unusable data include hemolysis of blood samples and concomitant GPIIb/IIIa antagonists.

It is unclear whether more extensive training would have produced higher data standards. Notably, the error rate was not lower in sites with more patients, where the assay was performed more frequently (Figure 5). Nor was the requirement for multiple attempts at validation strikingly associated with eventual site data

quality (not shown). Rather, a key factor influencing aggregation data quality is the commitment of the study site. Operators at several sites produced error-free sets of platelet aggregation data, and others had minimal issues. Among these were many not experienced in performing platelet aggregation, and some with no laboratory experience at all. While some sites eliminated certain classes of error based on feedback from the data reviewers, other sites made multiple errors and failed to improve despite prompting. Central to this difference is the dedication, conscientiousness, and responsiveness to feedback of operators and other site staff. A poor usability rate for platelet aggregation data might call into question the validity of all the platelet aggregation and other clinical trial data generated at such sites. However, no policy or procedure was in place in this study to exclude data from any site on this basis. Criteria to exclude results from sites consistently producing poor-quality data could be included in future trial protocols. In this study, the usable data from such sites are consistent with results from other sites, and so contribute to the analysis of the effect of the study drug on platelet responses.

We would suggest that for trials involving specialist assays, the local CRO responsible for choosing trial sites should be familiar with the assay requirements and effectively communicate to sites prior to initiation the burden such substudies place on site personnel, time, and other resources. A more stringent validation process could also help to reduce the number of sites producing unsatisfactory amounts of usable data.

In this study, electronically recorded data were burned onto CD and physically transferred for review. This delayed data review by up to 2 weeks or more, resulting in the repetition of some easily avoided errors. Secure electronic data transfer would rectify this shortcoming, allowing real-time review of data, more effective feedback to operators, and further improvements in data quality. This would also allow rapid return of quality-controlled data to the sponsoring pharmaceutical company at trial completion, shortening the time to submission to regulatory authorities. The review process described here is being developed along these lines.

CONCLUSION

With suitable site selection, training, and support, platelet aggregation can be performed in trial sites with no previous experience of the procedure. Independent review and monitoring of platelet aggregation objectively flags artifacts in the data, ensuring that high-quality data are produced for the study sponsor. Where quantitative platelet aggregation data are used as a study endpoint, this will allow the use of smaller group sizes or reduce uncertainty about the results.

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