Cardiology in the Young

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Efficacy and speed of effect after the first dose of aspirin in children with congenital heart disease

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Original Article

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Abstract

Background: Many paediatric studies report that patients must be established on aspirin therapy for a minimum of 5 days to achieve adequate response. This is not always practical especially in critical settings. Prospective identification of patients that are unresponsive to aspirin sooner could potentially prevent thrombotic events. *Aims:* The aim of this study was to investigate prospectively if the first dose of aspirin is effective in decreasing platelet aggregation, and thromboxane formation and if this can be measured after 2 hours in paediatric cardiology patients. A secondary aim was to identify a cut-off for a novel marker of aspirin responsiveness the maximum amplitude with arachidonic acid, which could potentially dramatically reduce the blood volume required. Third, we aimed to prospectively identify potentially non-responsive patients by spiking a sample of their blood *ex vivo* with aspirin. *Results:* The majority (92.3%) of patients were responsive, when measured 2 hours post first dose of aspirin. Non-response or inadequate response (7.7%) can also be identified at 2 hours after taking the first dose of aspirin. Additionally, we have shown a novel way to reduce blood sample volume requirements by measurement of the maximum amplitude with arachidonic acid as a marker of response, particularly for monitoring. *Conclusions:* These findings of rapid efficacy in the majority of patients offer assurance in a sound, practical way to attending clinicians, patients, and families.

Numerous studies in the paediatric population report that patients must be established on aspirin for a minimum of 5 days prior to assessing whether they demonstrate responsiveness using in vitro testing methods.[1–5] However, in paediatric cardiology, there are several settings where this is not practical. For example, in the post-operative period, thromboprophylaxis is essential and in many patients is provided by aspirin monotherapy. Furthermore, patients attending for day-case catheterisation procedures may be discharged on aspirin the same day. Demonstrating adequate inhibition of platelet aggregation following the first dose of aspirin would provide clinicians with clinical reassurance of adequate prophylaxis. Furthermore, the early identification of failure to respond to aspirin would allow treatment to be tailored accordingly potentially avoiding thrombotic events.

Non-response to aspirin can be characterised as pharmacokinetic or pharmacodynamic. Pharmacokinetic non-responsiveness is caused by insufficient plasma concentrations of aspirin in order to completely inhibit platelet aggregation despite adequate intake. Reasons include increased metabolism or clearance, increased platelet turnover, drug interactions, and bioavailability. Pharmacodynamic non-responsiveness will occur when there is adequate plasma concentration of aspirin; however, due to COX-1 genetic polymorphisms, the aspirin is unable to effectively inhibit platelet aggregation.[6] It has been also proposed that pharmacokinetic or pharmacodynamic non-response can be distinguished by spiking a sample of the patient's blood *ex vivo* with aspirin, in order to simulate a higher dose and to rule out pharmacokinetic resistance.[6–8] Predicting if a higher dose of aspirin results in aspirin responsiveness would be hugely beneficial in a paediatric high-risk population. Prospective identification of patients that are unresponsive to anti-platelets agents such as aspirin could potentially prevent thrombotic events by identifying these patients sooner.[6]

The primary aim of this study was to investigate prospectively, if the first dose of aspirin at an empirical dose of 3–5 mg/kg (up to a maximum of 75 mg) is effective in decreasing both platelet aggregation and thromboxane formation when measured at 2 hours post first dose of aspirin in paediatric patients with cardiac conditions. There is a paucity of data in paediatric patients regarding optimal test-timing. The limited data largely come from post-operative series,[9] the results of which may be challenging to interpret given the significant impact of cardiopulmonary bypass on platelet turnover and subsequent aspirin response.[10] Most published work on test

timing therefore comes from series of healthy adults.[1,11] Perneby et al. reported that platelet inhibition differs depending on whether sample collection was at the early or late stages of the 24-hour period.[12] Henry et al. have postulated that platelet TXA2 synthesis is fully inhibited within 1–2 hours post aspirin intake.[13] Therefore, we hypothesise that testing at 2 hours post aspirin administration can be used as an accurate marker for response in children.

Several tests of aspirin response were utilised in this study to ensure consistency in reporting. thromboelastography with platelet mapping is one such test that requires approximately 2.6 ml of whole blood to perform. One of the necessary intermediary parameters for thromboelastography with platelet mapping calculation is the maximum amplitude induced by arachidonic acid. The authors hypothesised that maximum amplitude with arachidonic acid could be utilised as a standalone test of aspirin responsiveness which would result in a 70% reduction in phlebotomy requirements, rendering it highly useful in paediatrics particularly in small infants. The secondary aim was to evaluate maximum amplitude with arachidonic acid when compared to gold standard measures of aspirin responsiveness. Third, we aimed to prospectively identify patients that are non-responsive by spiking a sample of their blood *ex vivo* with aspirin. To ensure robustness, we employed methods that directly measure the inhibition of platelet COX-1 activity and generation of thromboxane (TXA2) by aspirin.[1,14]

Table 1. Patient demographics of aspirin cohort

Characteristic	n = 13
Age, days	2708 (579–6506)
Years	7.4 (1.6–17.8)
Females, % patients	54%
Weight (kg)	24.5 (9.5–72.4)
Dose (mg/kg/day), mean ± SD	2.59 ± 1.43
Dose (mg/kg/day), median (range)	3.06 (1.03–5.03)
Diagnosis, % patients	
ASD	61.5
VSD	15.4
Tetralogy of Fallot	15.4
Portosystemic shunt	7.7
Procedure, % patients	
ASD device closure	61.5
VSD closure	15.4
RVOT stent	15.4
Partial device occlusion of shunt	7.7

Values are mean ± SD, Median (range), n (%), unless otherwise stated.
ASD = atrial septal defect, RVOT = right ventricular outflow tract, VSD = ventricular septal defect.

Materials and methods

Study design

The study conforms with the principles outlined in the Declaration of Helsinki and was approved by The Research and Ethics Committees of the Children's Health Ireland at Crumlin, Dublin, Ireland as a prospective observational study. This study formed part of a larger study on aspirin response in paediatric patients with congenital and acquired heart disease. All subjects were children and infants attending the National Cardiac Centre, Children's Health Ireland at Crumlin, Dublin, Ireland, between January 2022 and May 2023. Parental consent and assent (where applicable) were received for all participants. The treating clinicians were blinded to the results of the study, and the dose of aspirin was not adjusted for the purposes of this investigation.

Patients and healthy subjects

Patients eligible for enrollment in the study were ≤18 years of age with CHD scheduled for interventional cardiac catheterisation and were due to receive their first dose of aspirin following their procedure. Patients included those requiring stent implantation, device closure of atrial or ventricular septal defects and requiring aspirin therapy for 3–6 months. Exclusion criteria included use of other anti-platelet therapies or medications known to affect platelet function; anticoagulant use; major surgery within 3 weeks of enrolment; malignancy or haematological disease; and thrombocytopenia. Patients were prescribed aspirin at an empirical concentration of 3–5 mg/kg/day up to a maximum dose of 75 mg/day. Patients were admitted to the cardiac day unit for interventional cardiac catheterisation procedures as outlined in Table 1. Baseline samples were taken before the procedure and then 2 hours after aspirin initiation. A sub-group had samples also taken after their cardiac catheterisation procedure, before aspirin administration to determine any surgical effects.[15,16]

Healthy age-matched children attending the innocent heart murmur clinics confirmed with normal cardiovascular status served as a control group for comparison with the aspirin group. This cohort was also used to determine the results using the different platelet assessment methods among patients not taking aspirin. This control group stratified into six age categories with twenty patients in each (n = 120) was not taking any drugs known to affect platelet function. Baseline patient results were compared to this control cohort. To avoid any interference from conditions known to affect platelet production, we excluded patients with a malignant or haematological disease, or thrombocytopenia.

Blood was sampled at baseline and two hours post aspirin administration when the patient was post-procedure. Each patient received a single dose of aspirin (3–5 mg/kg up to a maximum of 75 mg). At baseline, tests comprised serum thromboxane B2 (TXB2), platelet light transmission aggregometry (LTA-AA), Thromboelastography Platelet Mapping (TEG-PM), C-reactive protein (CRP), coagulation screen and, full blood count (FBC). Two hours after the administration of aspirin, the above tests were repeated. If any of the patients showed poor response, they were advised that they would be repeated post 1 week of treatment with aspirin. *Ex vivo* addition of aspirin was performed on the baseline samples to see if this could be used to predict aspirin response and, any patients that were found to have sub-optimum response to aspirin to investigate the possible mechanisms of poor response.

Clinical data collection

Patient demographic and clinical data were collected in the cardiac day unit including gender, date of birth, weight, cardiac diagnosis, genetic syndrome, medications, patient history of thrombosis, or bleeding. Data also included the prescribed aspirin dose. All patients had a recent echocardiogram.

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Full blood count

Full blood count analysis included measurement of cell counts, haematocrit, and platelet indices and was performed using the Sysmex XN20 haematology analyser (Sysmex, Kobe, Japan). Platelet indices included platelet count, platelet distribution width, mean platelet volume, and immature platelet fraction. High immature platelet fraction, mean platelet volume, and platelet distribution width have been shown to be correlated with reduced aspirin response.[17] All samples were analysed within 2–4 h after sample collection.

Serum thromboxane B2

All platelet function results were correlated with serum thromboxane B2 results to measure adherence to aspirin. The thromboxane metabolite was measured in serum to obtain a pharmacologically specific measure of aspirin- induced COX-inhibition. Whole blood non-anticoagulated blood was incubated at 37°C for 1 hour, resulting in thrombin generation, platelet activation, and in vitro release of TXA2, followed by clot formation. Serum was separated by centrifugation at 2650 g for 10 minutes and stored at −80°C. Serum thromboxane B2 levels were later measured by using a commercial enzyme immunoassay (R&D Systems, Abingdon, U.K.).

Platelet thromboxane

As described by Maree et al., if incomplete aspirin inhibition of platelet COX is present, addition of its substrate, AA, results in the production of large amounts of platelet thromboxane (TX) resulting in a sensitive aspirin assay. In order to determine the presence of uninhibited platelet COX, we evaluated the amount of thromboxane B2 produced when AA was added to platelet-rich plasma, referred to as "platelet thromboxane".[8]

Platelet light transmission aggregometry

Platelet aggregation studies using light transmission aggregometry, and the agonist arachidonic acid were performed within 4 hours of blood collection in 3.2% citrate. Because smaller volume samples were taken for these studies than for routine diagnostic studies performed in the diagnostic laboratory, each stage was validated using the diagnostic laboratory as the reference method. Following optimisation whole blood was centrifuged at 170 g for 5 minutes and platelet rich plasma was collected. The remaining blood was spun at 2650 g for 10 minutes to obtain platelet poor plasma. Aggregation was performed using a Helena Biosciences Aggram aggregometer (Helena Biosciences UK) and using arachidonic acid at two concentrations 1 mM, and 1.64 mM as there is no consensus for the optimum concentration.[8,13,18–21] Samples where significant residual platelet aggregation was detected post aspirin intake were further spiked *ex vivo* with aspirin and reanalysed.[7,8] A sub-group of samples were further analysed for uninhibited platelet COX as described above.

Thromboelastography with platelet mapping

A platelet mapping assay (thromboelastography with platelet mapping) using the TEG® 5000 analyser platform was used to evaluate platelet aggregation and inhibition. The analysers and reagent kits are manufactured by Haemoscope Corporation (Niles, Illinois, United States). Analysis was performed according to manufacturer's instructions (Haemonetics Corporation, 2016). The percentage of platelet inhibition using the arachidonic acid agonist was calculated by TEGPM software as [100 − {(MA AA – MA Fibrin) /(MA Thrombin – MA Fibrin) × 100}] The MA is maximum amplitude and represents the strength of the clot, and represents total platelet activation using the citrated sample (MA Thrombin), an expression of fibrin only (MA Fibrin), and the presence of aspirin reflected in the reduction in maximum amplitude induced by arachidonic acid. The same software was also employed to calculate the percentage residual platelet aggregation as 100-platelet inhibition (%). Blood samples were analysed within 2.5 hours of collection. All samples were measured in duplicate and only reported when results were consistent. Repeated samples were analysed on any patients showing reduced response to aspirin. The initial and repeated samples were spiked *ex vivo* with aspirin to further investigate and predict aspirin responsiveness.

Maximum amplitude induced by arachidonic acid

The maximum amplitude (MA AA) represents the contribution of platelets not inhibited by aspirin resulting in a negative correlation between maximum amplitude induced by arachidonic acid and platelet inhibition. This is part of an overall calculation as described above in thromboelastography with platelet mapping which requires two samples comprising of 1.4 ml sodium citrate, 1.2 ml lithium heparin. Using the maximum amplitude induced by arachidonic acid alone would require only lithium heparin, a valuable solution in neonatal and paediatric care. This parameter was evaluated as part of the study. A surrogate AA (Helena Biosciences) (1 mM) was also employed as quality assurance. Volod et al. reported that the maximum amplitude induced by arachidonic acid parameter correlated better with aggregometry than the overall thromboelastography with platelet mapping and arachidonic acid percent inhibition parameter.[22]

Ex vivo spiking of samples

As a measure of predicting aspirin response plasma or whole blood samples pre aspirin and post-aspirin (where significant residual platelet aggregation or reduced inhibition was detected) were spiked *ex vivo* with aspirin and re-assayed within 1 hour with the test used initially.[7,8]

Blood collection

Blood sampling was performed in accordance with the BSH Guideline (Gomez et al., 2021). Venous or arterial blood was collected from each patient by sterile venipuncture directly into blood sample tubes. The first 1.5 ml of blood were collected in a tube without anticoagulant for serum thromboxane B2 measurement. Samples for analysis of full blood count were collected in 1.6 ml tubes containing EDTA K3E (Sarstedt, Germany). Samples for analysis of thromboelastography with platelet mapping were collected in 1.4 ml tubes containing 0.109 M sodium citrate (Sarstedt, Germany), and 1.2 ml tubes containing liquid lithium heparin (Sarstedt, Germany) for thromboelastography with platelet mapping. Sampling was coordinated with that of clinical venipuncture where possible. Given the small circulating volume in children and infants blood sampling was limited according to age. The remaining lithium heparin and sodium citrate samples were used for c-reactive protein and coagulation screen analysis, respectively.

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Adherence

Non-adherence was ruled out in this study as patients were given the aspirin in an observed setting. Plain aspirin was prescribed.

Aspirin non-response (resistance) definitions

There is no manufacturer recommended ranges for any of the parameters as outlined above or cut-offs for aspirin response in paediatric patients. Likewise, there is no formal definition for aspirin non-response based on serum thromboxane B2 in adults or children. Frelinger et al. used 3.1 ng/ml as it correlated with subsequent major adverse cardiovascular events,[23] other authors have used 2.45 ng/ml as the cut-off,[13] or a lower value of 2.2 ng/ml.[8] We used the lower threshold as described by Maree et al., with a plan to describe results up to 3.1 ng/ml as borderline. To add further strength to the results, we also used percent inhibition from the baseline as a cut-off. High levels of serum thromboxane B2 inhibition have been proposed to have clinical efficacy for aspirin, less than ninety-five percent inhibition of COX is defined as treatment failure and suggested to be the cut-off for non-response,[24] and incomplete thromboxane B2 inhibition as<99% inhibition.[1] In relation to light transmission aggregometry with arachidonic acid, studies have defined non-response as platelet aggregation above 20% using arachidonic acid.[25] Whilst in thromboelastography with platelet mapping, adult studies and some paediatric studies define a response to aspirin as greater than 50% inhibition, a partial response as 30–50% inhibition, and lack of response as less then 30% inhibition (Gurbel et al., 2007). The laboratory criteria applied in this study for aspirin non-response was platelet inhibition below 50%, semi response 30–50% inhibition by thromboelastography (thromboelastography with platelet mapping) and light transmission platelet aggregationabove 20% using arachidonic acid agonist. A category of semi-response was not applied in the case of LTA measurements. thromboelastography with platelet mapping data were confirmed using an extra surrogate AA (Helena Biosciences) agonist which was the same as that employed in light transmission aggregometry with AA assay.

Statistical analysis

The baseline results were compared to an age-matched control group as part of the overall study. Results of laboratory assays that were normally distributed in patients pre-aspirin dose (baseline) and post-aspirin are reported as mean + standard deviation. Median (range) was also reported to illustrate the range of data. Discrete variables were reported as frequency and percentages. Continuous variables were compared using the Student-t test and Pearson's chi-squared test or Fischer's exact test for discrete data. Analysis of variance was also used to analyse differences between pre-op, pre- aspirin, and post aspirin cohorts, a Kruskal–Wallis analysis of variance was used if the data were not normally distributed. All hypothesis tests were performed with two-sided tests. Pearson's correlation coefficient was calculated for the relationship between dose concentration and each of the platelet inhibition and aggregation parameters. All statistical analyses were performed using SPSS version 26 (IBM Corporation, New York, NY, United States of America) with a p-value less than 0.05 considered statistically significant.

Results

A total of 15 patients that were scheduled for interventional cardiac catheterisation procedures and were due to receive their first dose of aspirin post procedure were recruited to the study. Thirteen patients had complete data which are presented.

Patient demographics and laboratory characteristics

Table 1 shows the demographics of the overall patient population (n = 13). The median age was 7.4 years and comprised seven girls, and six boys. The most common single diagnosis and procedure was atrial septal defect (61.5%) and atrial septal defect device closure (61.5%) respectively. The baseline laboratory results were compared to an age-matched control group as part of the overall study with no significant difference. The mean first dose of aspirin was 2.59 ± 1.43, median 3.06 (1.03–5.03) mg/kg and 77% of participants were on the maximum dose of 75 mg/day and the remainder on half a tablet (37.5 mg/day). A sub-group had samples also taken after their cardiac catheterisation procedure, before aspirin administration to determine any surgical effects and we found no significant difference between pre-procedure and post-procedure results. We found good agreement between the assays for both baseline and 2 hours post aspirin intake measurements. In summary, two hours after administration of 3–5 mg/kg plain aspirin, platelet aggregation by thromboelastography with platelet mapping and arachidonic acid, light transmission aggregometry with arachidonic acid, and thromboxane formation were significantly decreased to a median of 15.7 (10–65.8)%, 16.5 (20.0–31.2)%, 0.38 (0.02–8.64)% of the initial values respectively. There was no significant difference found in the full blood count platelet parameters (including IPF)), C-reactive protein, and coagulation results between the pre and post aspirin measurements (Table 2).

Serum thromboxane B2 determination

Two hours after aspirin intake, the overall serum thromboxane B2 was reduced significantly to 1.02 ng/ml (mean ± SD: 1.02 ± 6.85 ng/ml) and inhibition of thromboxane B2 production by 99.0 (±2.23)% in 13 patients indicating low thromboxane B2 generation concordant with the effect of aspirin on the platelets (Table 3, Fig. 1). One patient (7.7%), who was also the youngest participant, had a thromboxane B2 level of 25.5 ng/ml resulting in just 91% inhibition of thromboxane B2 meeting our predefined criteria for treatment failure. When this patient was excluded from statistical analysis the mean serum thromboxane B2 further reduced to a mean of 0.77 ng/ml (±0.60) and median 1.07 (0.18–2.1) ng/ml with the maximum value of 2.1 ng/ml. This resulted in very consistent and strong thromboxane B2 inhibition results of 99.6 (+0.23), median 99.6 (99.2–99.9)%.

Platelet thromboxane

The patient that could be classified as having treatment failure by serum thromboxane B2 had a high residual platelet thromboxane at 120 ng/ml indicating that not all thromboxane B2 was inhibited by the aspirin (Table 3). This is elevated in comparison to a subgroup of patients with serum levels below the thromboxane B2 threshold that had a mean platelet thromboxane of 1.5 (±0.2) ng/ml.

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Table 2. Laboratory characteristics of the aspirin cohort

Laboratory characteristics	Pre-Op	Pre- ASA	2 h Post ASA	P-value
No.	13	5	13	
Haemoglobin (g/l)	122 ± 14.1	120 ± 15.1	124 ± 12.9	0.661
HCT (%)	35.4 ± 4.0	34.4 ± 3.8	35.5 ± 3.9	0.646
WCC (10^9/l)	6.9 ± 3.1	8.5 ± 2.9	6.6 ± 3.3	0.054
Platelets (10^9/l)	224 ± 79.0	232 + 75.5	221 ± 58.5	0.987
MPV (fl)	10.06 ± 1.33	10.05 ± 1.48	10.08 ± 1.03	0.965
PDW(fl)	10.98 ± 2.40	10.97 + 2.47	10.93 ± 2.33	0.981
IPF (%)	1.65 ± 1.40	1.85 ± 1.59	1.68 ± 1.35	0.972
IPF (%)	1.2 (1.0–4.1)	1.3 (0.8–4.2)	1.2 (0.7–3.6)	0.972
Reticulocytes (10^9/l)	59.1 ± 5.0	60.9 ± 5.6	59.4 ± 7.0	0.743
CRP (mg/l)	<5	<5	<5	0.999

ASA = aspirin; WCC = white cell count; MPV = mean platelet volume; PDW = platelet distribution width; IPF = immature platelet fraction.
Mean ± SD, median (range).
P-value; pre-op compared with post-dose, pre-dose compared with post-dose.

Table 3. Thromboxane measurements of the aspirin cohort

TXB2	Pre-Op	2 h Post ASA	P-value
	13	13	
TXB2 (ng/ml)	286.3 + 21.6	1.02 ± 6.85	<0.001
TXB2 (ng/ml)	295 (249–310)	1.07 (0.18–25.5)	<0.001
TXB2 inhibition (%)		99.03 ± 2.32	NA
TXB2 inhibition (%)		99.62 (91.36–99.98)	NA
PLT TX (ng/ml)	267.1 + 21.5	4.5 ± 59.2	<0.001
PLT TX (ng/ml)	287 (240–309)	1.7 (1.3–120)	<0.001

ASA = aspirin.
Mean ± SD, median (range).
P-value, pre-op compared with post-dose.
NA = not applicable.

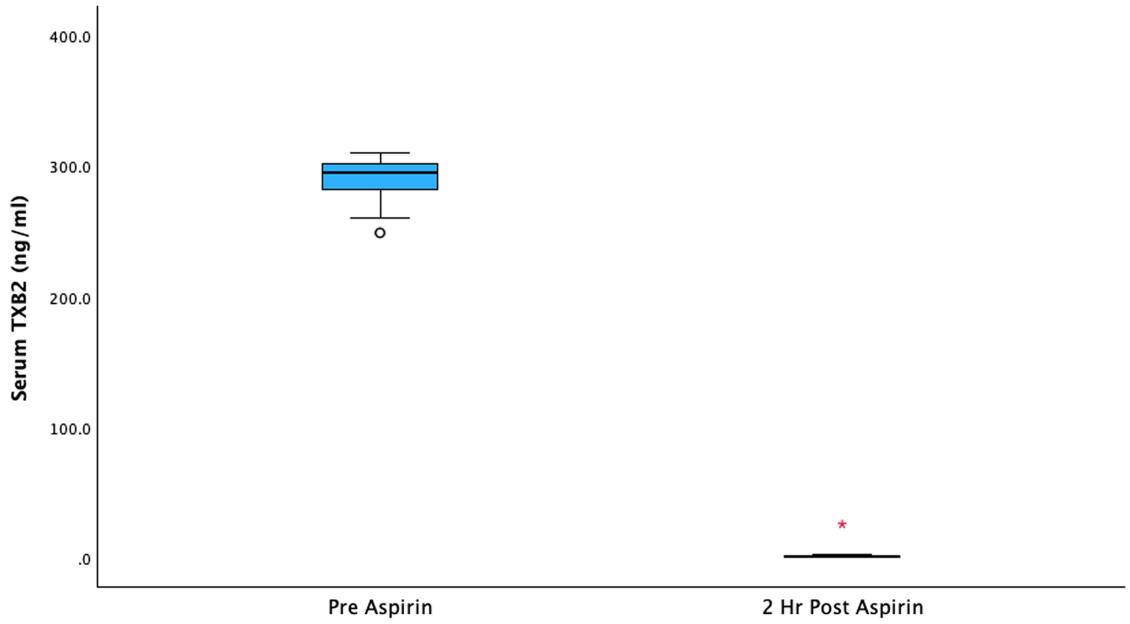

Figure 1. Serum thromboxane (TXB2) levels pre and 2 hours post-aspirin intake. * represents the patient with TXB2 above the threshold of 2.2 ng/ml.

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Table 4. Light transmission aggregometry using arachidonic acid (LTA-AA) results in the aspirin cohort

	Pre-Op	Pre-ASA	2 h Post ASA	P-value
No.	13	5	13	
LTA-AA (%)	93 ± 5.0	97.4 ± 6.4	18.7 ± 21	<0.001
LTA-AA (%)	95 (80–97)	100 (84.4–100)	15.7 (0–65.8)	<0.001

ASA = aspirin.
Mean ± SD, median (range).
P-value; pre-op compared with post-dose, pre-dose compared with post-dose.

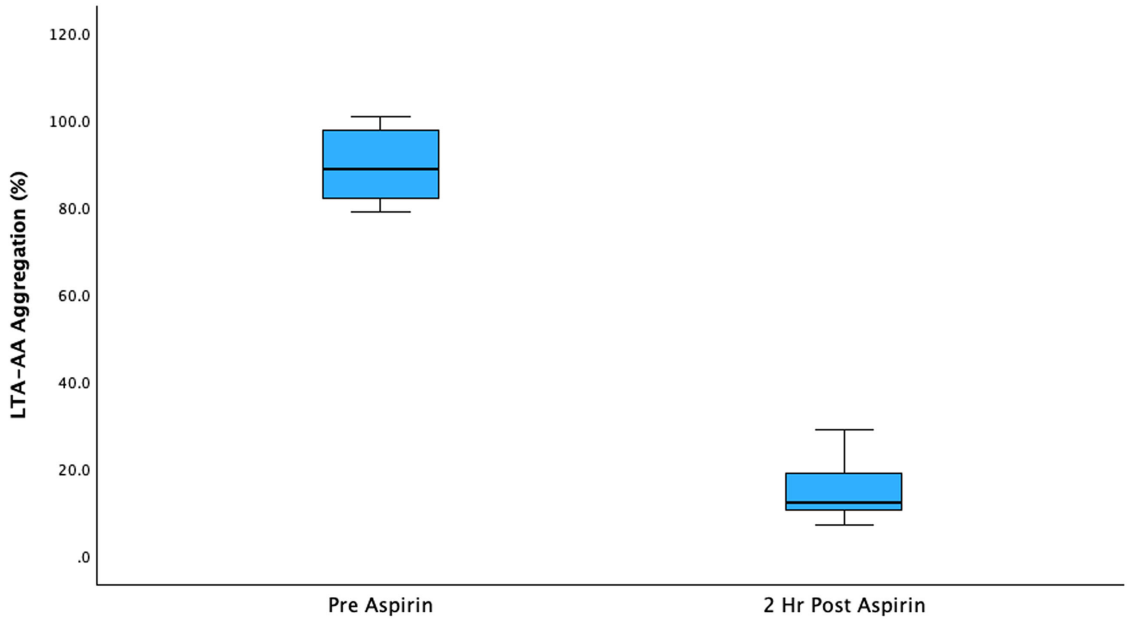

Figure 2. Platelet aggregation measured using LTA-AA pre and 2 hours post-aspirin intake.

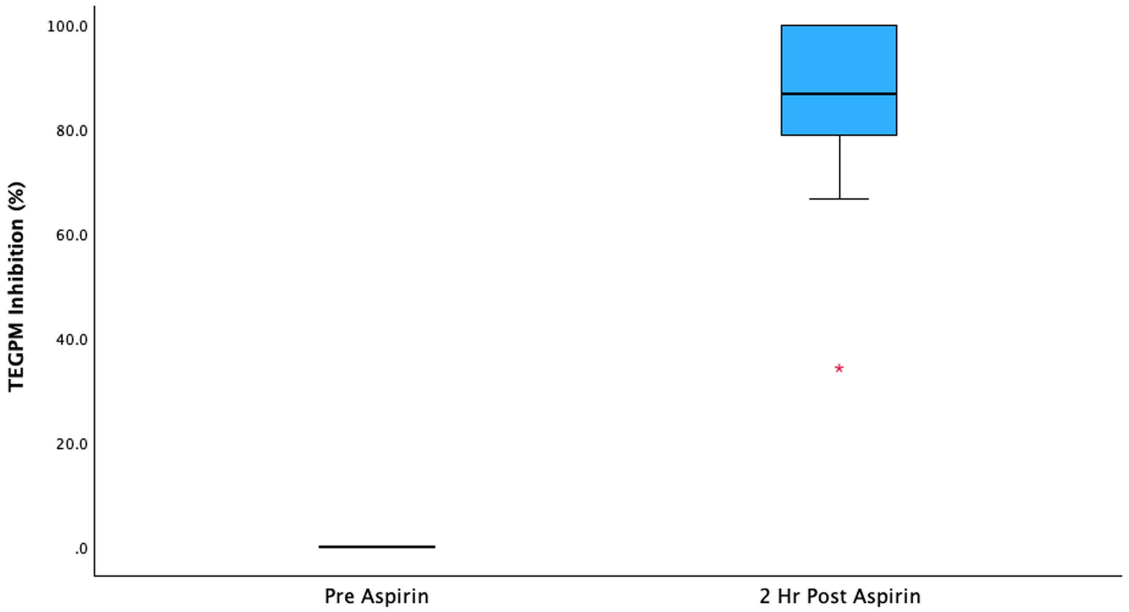

Figure 3. Platelet inhibition measured using TEGPM-AA pre and 2 hours post-aspirin intake. * represents the patient with TEGPM inhibition below the threshold of 50%.

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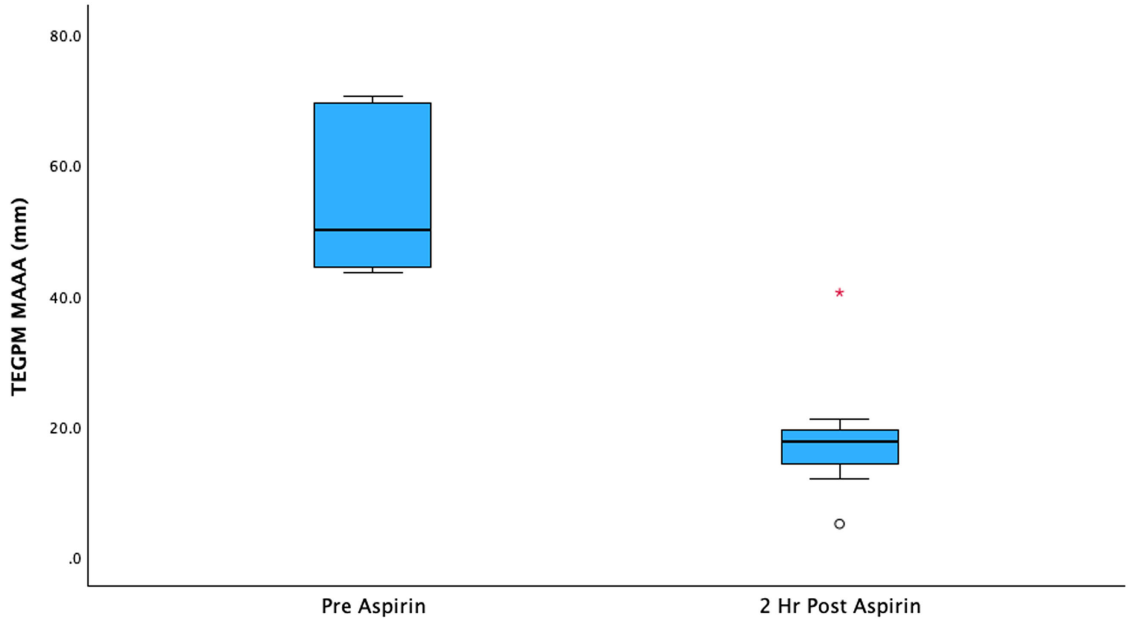

Figure 4. Platelet inhibition measured using TEGPM-AA and showing specifically the maximum amplitude parameter (MA AA) pre and 2 hours post-aspirin intake. * represents the patient with TEGPM inhibition below the threshold of 50% with MA AA of 40.5 mm.

Light transmission aggregometry with arachidonic acid

There was less than 5% non-significant difference between results using 1 mM and 1.64 mM and results are displayed for 1 mM (Table 4). Two hours after aspirin intake, the overall platelet aggregation had reduced from a mean baseline of 93 ± 5% to 18.7 ± 21% in the 13 participants (Fig 2). Again, the same patient showed a partial response in this assay whereby their platelet aggregation was reduced from 80% aggregation prior to aspirin intake to 29% but not sufficiently according to the threshold of 20%. Similar to the previous assays when this patient was excluded the mean platelet aggregation 2 hours after aspirin intake was observed to be reduced to 12.6 (± 4.5), median 11.5 (7–19)% an indication of the strong inhibitory effect of aspirin on platelet aggregation as measured by LTA-AA.

Thromboelastography with platelet mapping and arachidonic acid

Two hours after aspirin intake, the overall platelet inhibition was 81.3 (mean ± SD: 81.3 ± 20.9)% and median 84.3 (34.2–100)% in 13 patients indicating low platelet aggregation again consistent with the effect of aspirin on the platelets (Table 5, Fig 3). The same patient as above had a lower inhibitory response of 34.2%, below the threshold of 50% and was classified as semi-responsive according to our criteria. When this patient was excluded from calculations the mean platelet inhibition further increased to a mean of 87.7 (±12.6), median 93.4 (66.7–100)% with a minimum of 66.7% inhibition. We introduced the same AA agonist as that used in LTA and analysed another aliquot. Results were consistent with the thromboelastography with platelet mapping and arachidonic acid but showed stronger correlation with light transmission aggregometry with arachidonic acid.

Maximum amplitude induced by arachidonic acid

To evaluate the maximum amplitude induced by arachidonic acid parameter, we observed that aspirin reduced the maximum amplitude with arachidonic acid from a mean baseline of 55.6 ± 11.0 mm to 17.0 ± 7.5 mm (Table 5, Fig 4). Again, excluding the patient that had an inadequate response when full thromboelastography with platelet mapping and arachidonic acid criteria was applied the mean maximum amplitude with arachidonic acid was 15.0 mm (±7.5). Consistent with published recommendations, we considered serum thromboxane B2 to be the most definitive marker of aspirin response, because aspirin specifically inhibits COX-1 and therefore directly inhibits the generation of TXA2, and its stable metabolite thromboxane B2.[1,2] Using thromboxane B2 as the gold standard this would suggest <30 mm (15.0 ± 15, mean ± 2SD) as a cut-off for aspirin response for maximum amplitude induced by arachidonic acid. Application of same methodology for cut-off calculation resulted in a threshold for light transmission aggregometry with arachidonic acid of 21.6 % (12.6 ± 4.5 mean ± 2SD).

Aspirin spiking

All samples were spiked *ex vivo* with aspirin and showed platelet inhibition. The patient with treatment failure as defined by thromboxane B2 inhibition had a lower than initial platelet thromboxane B2 (12 ng/ml) after spiking but remained with a higher platelet thromboxane than other patients.

Summary of patient with reduced response

The patient with treatment failure as defined by thromboxane B2 inhibition was the youngest in the cohort at 1.6 years (579 days), all other patients were greater than 2 years of age. The patient was prescribed 3.94 mg/kg/day, the mean dose of the overall cohort was 2.59 ± 1.43, median 3.06 (1.03–5.03) mg/kg. The patient had a primary diagnosis of ASD and ASD closure like seven other patients. All methodologies defined this patient as having partial or sub-optimum response. Results of this patient were similar when repeated at 7 days post dose, this patient continued to display a

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Table 5. Thromboelastography and platelet mapping with arachidonic acid (TEGPM-AA) inhibition, and maximum amplitude (MA AA) results in aspirin cohort

	Pre-Op	Pre-ASA	2 h Post ASA	P-value
No.	13	5	13	
TEGPM AA INHIB (%)	2.0 ± 5	2.6 ± 6.4	81.3 ± 20.9	<0.001
TEGPM AA INHIB (%)	0 (0–10)	0 (0–15.6)	84.3 (34.2–100)	<0.001
MA AA (mm)	53.2 (46.6–70.5)	54.2 (50.0–69.5)	17.6 (5.0–40.5)	<0.001
MA AA (mm)	55.6 ± 11.0	56.6 ± 8.0	17.0 ± 7.5	<0.001

ASA = aspirin.
Mean ± SD, median (range).
P-value; pre-op compared with post-dose, pre-dose compared with post-dose.

sub-optimum response. However, ex vivo spiking studies promisingly predicted that this patient responded to a higher dose.

Discussion

The main findings in this study are as follows; in general patients responded well to aspirin, 92 % had satisfactory response and the remainder partial response. We have shown that the first dose of aspirin does work as observed in each of the tests where the inhibitory effect of aspirin was evident. Consistent with findings by Perneby et al., and Henry et al., in adult studies[12,13] we have shown in this paediatric study that the majority patients are responsive and this can be measured at 2 hours post aspirin dosing. Non-response or inadequate response can be identified at 2 hours after taking the first dose of aspirin. Although only one patient in this study displayed non-response at two hours (1/13), they had the same result following 7 days of aspirin intake.

The patient with partial response to aspirin was the youngest in the cohort at 1.6 years (579 days) and was prescribed 3.94 mg/kg/day. While it is challenging to draw concrete inferences from this small sample size, all other patients were greater than 2 years of age and were on a dose ranging from 1.05 to 5.03 mg/kg suggesting that younger children possibly require a higher dose of aspirin per weight as they do with other anticoagulants. This is consistent with aspirin studies in younger children.[26,27] All tests identified this patient with partial response as such. Consistent with published recommendations, we considered serum thromboxane B2 to be the most definitive marker of aspirin response because aspirin specifically inhibits COX-1 and therefore directly inhibits the generation of TXA2, and its stable metabolite thromboxane B2.[1,2] There was good agreement between the assays which was aided by using assays that specifically measure COX/thromboxane generation by aspirin[14] and additionally by using the same AA agonist as employed in light transmission aggregometry with arachidonic acid as an extra surrogate marker in thromboelastography with platelet mapping and arachidonic acid.

Aspirin response defined by serum thromboxane B2 showed good agreement with the defined aspirin thresholds in the other assays light transmission aggregometry with arachidonic acid (at both AA agonist concentrations), and thromboelastography with platelet mapping and arachidonic acid. These results were also confirmed by platelet thromboxane measurement and spiking ex vivo. We were able to demonstrate agreement between maximum amplitude with arachidonic acid and the other tests and the benefits of using a novel application of the maximum amplitude induced by arachidonic acid as a standalone test of aspirin responsiveness particularly for monitoring, which would result in a 70% reduction in phlebotomy requirements, rendering it highly useful in paediatrics particularly in small infants. Additionally, we defined the cut-off for light transmission aggregometry with arachidonic acid to be 21.6% (12.6 ± 4.5 mean ± 2SD) consistent with findings by other authors that have recommended a variety of AA agonist concentrations.[8,13,18–21] While ex vivo spiking is a crude method, it does have value in predicting pharmacokinetic resistance.[7] We observed in this preliminary study that aspirin response could be predicted using ex vivo spiking of the samples and suggesting that the patient with the sub-optimum response most likely required more aspirin.

Limitations

Not included in this study were neonates and patients following major cardiac surgery in whom rates of thrombotic events are elevated.[26,27] Neonatal patients have an immature haemostatic system, and rates of aspirin resistance in this group appear to be higher than reported rates in other age categories.[27,28] Furthermore, patients in the post-operative period, particularly after cardiopulmonary bypass, also demonstrate high rates of resistance potentially as a consequence of high platelet turnover.[10] This study instead consisted of stable paediatric patients attending for elective day case procedures. While this limits the generalizability of the results, it provides a pilot group on which larger work can be based. It also demonstrates when some of the mitigating factors outlined above are removed, that the majority of paediatric patients when prescribed aspirin in an observed setting were responsive when measured 2 hours post-dose. Further limitations include the small sample size, which again limits the conclusions that can be drawn from the data. However, assays and results have been confirmed with confirmatory methods. Also due to small numbers of patients with non-responsiveness, we were unable to construct an ROC curve to select a cut-off, sensitivity, and specificity for maximum amplitude with arachidonic acid. However, we were able to define a cut-off using the mean and two standard deviations. This holds promise as a method of assessing responsiveness in paediatric patients with a decreased phlebotomy requirement, although larger pilot studies are required. Similarly, we were able to predict a pharmacokinetic response to increased doses of aspirin, using ex vivo spiking of samples. However, we must caution that this was a small cohort with the majority of patients exhibiting a response to aspirin, it does, however, provide us with an algorithm for future studies. Finally, the patient with the sub-optimum response may have a faster clearance of aspirin and may require more frequent dosing rather than a higher dose. A previous study has suggested that usual once-daily aspirin dosing may be insufficient to adequately inhibit platelet aggregation in adult patients with an increased platelet turnover[11] and inadequate inhibition could be

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corrected using a twice-daily protocol,[29,30] we did not find any association between either inadequate response or platelet turnover, but our study numbers were too low to draw any such conclusions and would require further investigation. Evaluation of the dosing interval was also outside the scope of this study.

Conclusion

In summary, in this cohort we can predict aspirin response 2 hours after the intake of the first dose of aspirin in children. This finding would offer assurance in a sound, practical way to the attending clinician, the patient and their families. Additionally, we have shown a novel way to reduce blood sample volumes requirements by measurement of the maximum amplitude induced by arachidonic acid as a marker of response, particularly for monitoring. We have also demonstrated that aspirin response both baseline and escalated doses could be predicted using ex vivo spiking of samples. The algorithms applied hopefully provide a pathway for larger multicentre studies to characterise aspirin responsiveness in the greater paediatric population.

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