Measurement of the Association Between Mean Platelet Volume and Embolic Complications of Infectious Endocarditis

BY

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ABBREVIATIONS

AIDS: Acquired Immunodeficiency Syndrome
ClfA: Clumping Factor A
ClfB: Clumping Factor B
CKD: Chronic Kidney Disease
COPD: Chronic Obstructive Pulmonary Disease
CNS: Central Nervous System
DITP: Drug-Induced Immune Thrombocytopenia
fL: Femtoliters
HIV: Human Immunodeficiency Virus
IDU: Injection Drug Use
IE: Infective Endocarditis
IDU-IE: Injection Drug Use-Associated Infective Endocarditis
FnBPA: Fibronectin Binding Protein A
FnBPB: Fibronectin Binding Protein B
MAHA: Microangiopathic Hemolytic Anemia
MPV: Mean Platelet Volume
OE: Oxymorphone-Exposed Injection Drug Users
OSH: Outside Hospital
OU: Oxymorphone-Unexposed Injection Drug Users
PAAP: Platelet Aggregation Associated Protein
S. aureus: Staphylococcus aureus
Sdr: Serine Aspartate protein family
TTP: Thrombotic Thrombocytopenic Purpura
TV: Tricuspid Valve
vWF: von Willebrand Factor
WBC: White Blood Cell Count
WFBMC: Wake Forest Baptist Medical Center
ABSTRACT

**Problem:** Only a small number of low powered studies have assessed the relationship between mean platelet volume (MPV) and embolic disease of endocarditis. This work seeks to assess this association in the largest cohort to date and become the first to assess MPV in injection drug use associated endocarditis cases (IDU-IE).

**Methods:** Patient charts from 1/1/2004-9/31/2015 were manually reviewed. Only those who reported IDU in the 3 months prior to admission were classified as IDU-IE. IDU-IE cases were stratified into those that had injected oxymorphone (OE) and those who had not injected oxymorphone (OU).

**Results:** MPV is significantly associated with embolic disease of endocarditis even when demographics, clinical disease characteristics, and laboratory data are controlled for (OR 1.33 [1.07-1.64], p=0.002). Larger MPV at admission is associated with drug use (p=0.0004). Oxymorphone injection drug use was significantly more likely to be associated with MPV greater than or equal to the median of the reference range. (80.2% of the OE group vs 59.5% of the OU group, p= 0.048). There is no evidence that drug use interacts with or moderates the relationship of MPV with endocarditis embolic disease.

**Conclusion:** MPV is significantly associated with embolic disease of endocarditis independent of vegetation size and location.
CHAPTER 1: Background and Rationale

Historical Endocarditis

Osler himself described the entity of endocarditis in his Gulstonian lectures of 1885. What he went on to elucidate in these definitive lectures are the classic endocarditis epidemiology, disease presentation, and pathology. When we of modern times reference epidemiologic shifts or changes to historical presentation, it is to this baseline that we refer. Indeed, Osler’s sole purpose in giving this speech was to provide a synthesized and systematic review of academic evidence on ‘etiological, clinical, and anatomical characteristics’ of endocarditis that had been accumulated in the prior half century. The baseline definition of the disease was set here as ‘a disease of the lining membranes of the heart or its valves’. This could be a primary disease, usually in the infirm or those with preexisting heart lesions, or as a complication of other illnesses. On postmortem exam the lesions could be seen as vegetative growths, ulcerations, or suppurative lesions with deeper heart structures also involved. These lesions were known to be composed of fibrin, dead tissue, and ‘micrococci’. The left sided valves were affected most frequently, with right sided heart lesions seldom occurring in isolation. This was thought to be due to the low numbers of congenital cardiac lesions that occur on the right side of the heart and the lower right heart pressures contributing to less wear and tear of the tricuspid/pulmonic valves.

In addition to the cardiac lesions themselves, extracardiac phenomena were, and remain, common in endocarditis. It is the so-called ‘embolic phenomena’ that give classic endocarditis cases their most unique features. As the vegetation of bacteria builds upon the heart, pieces will break off to ‘embolize’ to various organs and the skin. These
result in microhemorrhages, pneumonia (right sided lesions), stroke, and major arterial occlusion (left sided lesions\textsuperscript{1-4}). They can even embed themselves within the walls of major blood vessels to form subsequent mycotic aneurysms\textsuperscript{5}. Over weeks of time, immunogenic phenomena can cause painful lesions on the skin called Osler’s nodes, dysfunction of the kidney (glomerulonephritis), and/or splenomegaly\textsuperscript{1,6,7}.

Osler and his contemporaries did not entirely understand why certain disease manifestations occurred in some cases but not others\textsuperscript{1}. Presentation differences were partly explained when further investigation revealed that the latter 2 phenomena of Osler’s nodes and glomerulonephritis occurred only in cases termed ‘subacute’\textsuperscript{6,7}. These were cases distinct from rheumatic or syphilitic endocarditis in both underlying cause and nature of the disease who lived for months or years rather than the few weeks of a patient with ‘acute’ endocarditis\textsuperscript{4}. The immunogenic lesions of the subacute cases could only form with time and a low pathogenicity organism the immune system could control but not fully eradicate. The immunogenic lesions are in contrast to the embolic lesions which can occur in any case at any time\textsuperscript{6,7}. This distinction between subacute findings and acute findings arose around the same time that it became evident that different strains of bacteria resulted in different disease presentations\textsuperscript{3}. Slowly the medical community began to understand the epidemiologic reasons underlying the vast heterogeneity of endocarditis cases.

In the early 1900s infective endocarditis (IE) was a disease of young adults, 60% males, with a large proportion having rheumatic heart disease as the predisposing illness\textsuperscript{4}. Exactly how often the subacute/chronic form was seen as compared to the acute form was difficult to pin down at the time in part due to terminology\textsuperscript{4}. Reports ranged from 8-40+
percent of cases with possible geographic variations. The vast majority of cases, especially those deemed ‘subacute’, were due to so called streptococci and in particular the group known as viridans streptococci, common mouth commensals. Despite the increasing level of insight in disease epidemiology and pathology, the disease remained universally fatal until the 1940s.

In the 1940s Penicillin emerged as treatment for endocarditis. The viridans streptococci were, and for the most part still are, highly sensitive to Penicillin. The first trials did not go well however- treatment was too short and delivered at too low a dose. It was not until treatments with higher doses of penicillin given over 6-8 weeks at a time were started that successes became widespread. Later studies would suggest that this need for long duration therapy is in part due to the fact that bacteria within large vegetations enter a protected resting state much less affected by antibiotics. Once the duration and doses were figured out, treatment failures at this point seemed more frequently due to delay in diagnosis and baseline poor cardiac function rather than drug resistant organisms, an entity uncommon at this time.

The Post Antibiotic Era of Endocarditis

The era of antibiotics has changed everything in this world and endocarditis is no exception. In the later 1940s and the 1950s reviews began appearing discussing a shift in the observed epidemiologic patterns of endocarditis. The average age of patients shifted from a younger group in their thirties to those in their 50s. The number of cases with rheumatic fever as a predisposition steadily declined as did those with preceding dental procedures. In their place new risk factors emerged of intravenous drug use (IDU), cardiac surgery and indwelling catheters, and the general sclerosis of valves that occurs
Somewhat ironically, a previous case of endocarditis could now be survived and this too proved to be a risk factor for future endocarditis. With the tradeoff in risk factors, incidence rates of endocarditis have been estimated to be surprisingly steady at 3-10 per 100,000 person years. The overall rarity of the disease has been a key limitation to fully describing cohesive, actionable trends in the disease process. Our view of what endocarditis looks like, how it behaves, and how it should be treated come from aggregated population studies conducted in highly heterogeneous settings and countries. With the varieties of disease presentation, complexity of case diagnosis, evolving case definition, and geographic population variability, conflicting results are often found.

One way of trying to reconcile these conflicts was to attempt to streamline and standardize case definitions. Osler foreshadowed the difficulty of nomenclature when he noted that the terms and ‘the forms of endocarditis are as numerous as the diseases in which it occurs’. The symptoms of this disease resemble those of any number of severe infections such as pneumonia or simple bacteremia but endocarditis requires a much more robust treatment. Thus accurate case definitions are important for both clinical and research purposes.

The first definition to gain some common use was the Von Reyn criteria of 1981. These were a step up in unifying diagnoses but had many problems. The only way to achieve definitive diagnosis was histology or culture from surgery or autopsy. Echo was an up-and coming technology in the 1970s and increasingly relied on for imaging of endocarditis, but the Von Reyn criteria did not include echo criteria for diagnosis. In 1994 the Duke Criteria were proposed and proved to have superior
sensitivity to the Von Reyn criteria as they incorporated echo criteria and accounted for high risk groups such as intravenous drug users\textsuperscript{18,21}. These criteria were updated in 2001 in an attempt to better distinguish possible cases from unlikely cases\textsuperscript{22}. Some minor criteria were modified and Coxiella and Staphylococcus aureus (S. aureus) were added to the list of major criteria organisms. Though complex, this remains the most widely used definition of endocarditis to date.

These criteria were not only late in coming, they also did not resolve as much disparity in case reporting as would be hoped. Two key epidemiologic issues have been raging for over 50 years with little change in the debate even since standardized definitions came into play. The first of these debated issues is the rise of Staphylococcus aureus as the predominant cause of endocarditis. S. aureus has been reported as a cause of endocarditis since the entity was first described\textsuperscript{1,3}. However, starting in the late 1940s and 1950s, reports suggested that these cases were becoming increasingly common\textsuperscript{9}. Epidemiologic surveys of the time noting the changing features of endocarditis that are described above also noted increases in Enterococci and Staphylococcus aureus\textsuperscript{9}. This shift in flora was not only due to the changing nature of the comorbidities predisposing to the disease (skin incisions with surgery predisposing to S aureus rather than dental infections predisposing to viridans streptococci) but also due to the fact that S aureus and enterococci were those gram positive organisms least sensitive to penicillin\textsuperscript{23}. Staphylococcus almost immediately became resistant to penicillin with low rates of cure compared to the streptococci; rates improved upon invention of the anti-staphylococcal penicillins in the late 1950s\textsuperscript{24-26}. 
Shifts in etiologic organisms have great bearing on the presentation and diagnosis of endocarditis. S. aureus is an aggressive pathogen with multiple virulence factors even without taking into account the added challenges of methicillin resistant S. aureus in the last 30 years. The virulence of S. aureus allows it to infect even previously normal valves or valves with minimal damage. The virulence also results in a rapid onset, acute illness with patients seeking care early in the disease course. This ironically makes them more difficult to diagnose as though S. aureus is particularly damaging to cardiac structures, this damage takes time to manifest itself in the form of new murmurs or embolic phenomena. What’s more, S. aureus is particularly adept at causing pyogenic infections at distant sites once it has made it to the blood stream regardless of whether the heart is involve; this further muddies the diagnostic waters as these can mimic embolic complications.

Over this time of change in the mid-century, many were willing to admit staphylococcus aureus caused a not insignificant portion of cases but it was hotly debated and unclear just exactly how much S. aureus was occurring. Some reports were seeing more dramatic rises than others. Case reviews from Cincinnati General Hospital from 1940-1971 and Presbyterian Hospital in NY City from 1938-1967 reported stable incidences. Meanwhile, Quinn and colleagues found a 6-fold increase from 1953-1962 at their hospital and Boston City Hospital found S. aureus has become the second most common organism following a rapid rise starting in the late 1940s. Series with higher numbers of staphylococcal endocarditis cases became more frequent in the 1970s and 1980s commensurate with increasing reports of IDU-related cases. By 2000 It was common enough that it was included in the major criteria organisms of the modified
Duke Criteria\textsuperscript{22}. The 2000s then saw the rise of epidemiologic case series reporting staphylococcus aureus as the primary cause of endocarditis, overthrowing the long reign of viridans streptococci\textsuperscript{33}.

S. aureus’ claim to the throne is still disputed. A case series of Olmstead county reviewing cases from 1970-2000 accounting for 102 analyzed IE episodes found no change in incidence and viridans streptococci cases continued to outnumber S aureus cases\textsuperscript{34}. It was suggested that disproportionate numbers of reports came from tertiary hospitals where patients had more comorbidities, lines, surgeries, and risk factors for endocarditis in the hospital setting, a setting where staphylococcus aureus is known to reside in higher amounts. There was also the possibility of referral bias with more acute S aureus cases possibly being referred to these centers more frequently.

The dominance of S. aureus is also less clear in other countries. A recent review of the literature as to causative agents found that viridans streptococci remain the predominant cause of endocarditis in Asia\textsuperscript{35}. The International Coalition on Endocarditis found that many Asian, African and S. American countries all still have more Streptococcal disease as compared to Staphylococcal\textsuperscript{36}. Finally, a rigorous systematic review of the epidemiologic literature showed that even among countries where S. aureus is the most prevalent, it is only in the United States that the prevalence of S. aureus related cases are increasing\textsuperscript{37}. Interestingly, this was associated with a significant rise in IDU-endocarditis cases in N. America until the last decade when the association lost strength.
**Injection Drug Use Associated Endocarditis**

This brings us to the second ongoing controversy: the distinct nature of IDU-associated endocarditis. IDU has long been recognized as a risk factor for endocarditis and other infections; medical case reports of endocarditis in intravenous drug users go back at least to the 1930s\(^3\). The incidence of endocarditis in this group, though difficult to assess, is much higher than in non-injectors with estimates up to 11.9 cases per 100,000 person years\(^3\). How common IDU-IE is varies widely across geography. Some endocarditis reports have had 60-75% of their endocarditis cases occurring in injection drug users\(^4\). Many others have 5-15%\(^4\). A recent international prospective endocarditis study found that among 11 centers sampled in the US with >500 IE cases, about 16% were IDU-related\(^3\). This was the highest of any other countries studied. Slipczuk’s systematic had the complementary finding of a statistically significant increase in IDU-related cases in N. America from 17.3% in the 1980s to 50.7% in the 2000s (CI 10.7-23.9, \(p<0.05\))\(^3\). A third study has echoed these findings by concluding that there is an increase in IDU-endocarditis\(^4\) but this particular paper is opposed by a data review using nearly identical sources that states the proportion of IDU-IE is stable while IE as a whole had increased\(^4\).

In the late 1940s and 1950s, reports first began to be published of distinct characteristics of endocarditis in injection drug users: markedly higher proportions of tricuspid valve (TV) endocarditis and S. aureus involvement\(^4,45\). This phenomenon was met with scrutiny. A relatively large series by Cherubin et al. from 1960-1967 of 36 IDU-endocarditis cases had TV involvement in 18% (9% sole TV, 9% in combination with another valve)\(^9\). While acknowledging that this is still larger than the 5-10% of TV cases seen in the non-injecting population, the authors proposed that the increased
frequency of TV reports were due to either reporting bias or regional variation in drug use as many of the previous cases reported were from the Washington DC area. Since this original debate in the literature many reports have gone on to find this TV valve predominance in IDU-cases, but not all. The reasons for these differences and their meaning remain unclear.

Given the variability in the IDU-endocarditis populations studied, it has also been difficult to determine overall mortality. If anything can be considered axiomatic in IDU-endocarditis, it is that the in-hospital mortality is significantly less than that of their non-injector counterparts. Non-IDU cases have on average, and with a wide range of variability, about 20% inpatient mortality. This is in contrast to IDU-cases where most studies report a mortality of <10%. It must be appreciated that ‘less’ mortality does not equate to ‘insignificant’ mortality, however, and some groups report more striking mortality rates. One series had an in-hospital mortality of 24%. In another review of 31 consecutive cases admitted to the ICU, the mortality was 26%. Thalme and colleagues, who had the standard inpatient mortality rates overall of <10%, found that on longer follow-up left sided IDU patients actually had the highest mortality rates, even slightly higher than that of their non-IDU counterparts. In the era prior to combined antiretroviral therapy, Human Immunodeficiency Virus (HIV) positive injection drug use patients had higher rates of endocarditis acquisition and mortality; outside of IDU, HIV status has not seemed to contribute to endocarditis risk. It is less clear in the modern era of well controlled HIV if HIV status drives morbidity and mortality as many studies no longer see differences in outcome.
Embolic Disease and Surgery in Endocarditis

Embolic disease, surgical intervention, and mortality are the most commonly discussed and reported outcomes of endocarditis. Embolic phenomena are common in endocarditis and a significant factor in morbidity and mortality\textsuperscript{61-63}. Right sided valves embolize to the lungs with frequent formations of cavitary pneumonia. Left sided valves can embolize through the body including to the eye, major arteries of the limbs and vasculature, the spleen and other organs, the coronary arteries, and the skin where janeway lesions and splinter hemorrhages aid diagnosis. Emboli to the brain are the most common; symptomatic neurologic events occurring in 20-40\% of cases\textsuperscript{64}. An even higher amount of asymptomatic cerebral emboli have been shown to occur with some studies citing up to 80\%\textsuperscript{65,66}. Their presence complicates surgical decision making as the vascular shifts, blood thinners, and potential increased embolization arising from the cardiopulmonary bypass itself may result in neurologic deterioration\textsuperscript{64}. Early studies suggested worse outcomes in those who had early surgical intervention, but more recent studies with more sophisticated statistical techniques have shown that the risk is not substantially different between early and delayed surgery in these patients\textsuperscript{64}. Surgical intervention in the setting of cerebral emboli remains an area in need of further research.

The uncertainty regarding surgical intervention in the setting of cerebral emboli reflects the debate about how the presence and risk of embolic phenomena as a whole should influence surgical decision making. The advent of echocardiography allowed visualization of vegetations in the 1970s and became more central to the diagnosis of endocarditis with its incorporation into the Duke criteria\textsuperscript{18,67}. The ability to see vegetations raised the possibility that physicians could surgically remove them and thus limit their damage. Unfortunately, emboli most commonly occur early in the disease
course with declining risk over time which places a time clock on the surgical decision for this indication. Acting quickly after diagnosis precludes observation of the disease course limiting the time to assess additional factors that influence surgical decision making\textsuperscript{68,69}. What is more, surgery for this indication then means operating upon and potentially placing prosthetic material in an actively infected field since the surgery would be occurring before complete antibiotic therapy. Infection of a prosthesis with recurrent endocarditis is more common in such instances\textsuperscript{70}. Attempts have thus been made to determine what, if any, vegetation characteristics constitute a high enough risk of embolization to the patient that the surgical risk is warranted. As expected, there have been discordant findings and recommendations. The 2009 European Society of Cardiology (ESC guidelines) recommend urgent surgery as a class I indication in patients with one or more embolic episodes and a vegetation >10mm in length despite appropriate antibiotic therapy; urgent surgery is recommended as a class IIb indication in patients with isolated, very large vegetations of >15mm\textsuperscript{71}. The 2014 American Heart Association/American College of Cardiology (AHA/ACC) guidelines have only a class IIb recommendation to ‘consider’ early surgery in patients with mobile vegetations greater than 10 mm in length\textsuperscript{72}. The 2015 American Heart Association (AHA) guidelines note that early surgery is ‘reasonable’ in: 1) patients with recurrent emboli and persistent/enlarging vegetations on therapy (class IIa, evidence level B), 2) those with severe valvular regurgitation and mobile vegetations >10mm (class IIa, B level of evidence) or 3) when there are mobile vegetations >10mm, especially when on the anterior leaflet of the mitral valve, and associated with OTHER relative indications for surgery (class IIb, level C evidence)\textsuperscript{73}.
These conflicting guidelines highlight some of the difficulties involved in surgical decision making in endocarditis. Data for surgical outcomes has faced the same challenges as epidemiologic studies: heterogeneous populations studied over many decades during which diagnostic, medical, and surgical advances rapidly developed. Surgery in the IDU endocarditis population is even more contentious.\textsuperscript{74,75} When the left sided heart valves are involved, IDU populations are generally treated along classical guidelines. However, tricuspid valve surgical indications are less well defined than those for classic, left sided endocarditis.\textsuperscript{73} Currently, there is some evidence that cases with especially large vegetations (>20mm) and persistent sepsis should be considered for early surgery in tricuspid endocarditis.\textsuperscript{73} Regardless of the valve involved, large concern exists about recurrent infections of prosthetic valves should substance abuse continue or relapse. Prosthetic valve infections tend to have more severe complications than native valves including higher likelihood of annular abscess and an overall mortality of 23-59\%.\textsuperscript{76,77} The risk for prosthetic valve endocarditis is higher in patients who undergo surgery during the active phase of the disease.\textsuperscript{76} Infections of prosthetic valves often require repeat surgery due to the difficulty of eradicating pathogens from a prosthetic surface and the increased rate of complications.\textsuperscript{76} Thus surgical treatment of an injection drug user with placement of a prosthetic valve for classical surgical indications could potentially result in worse long term outcomes as well as squander resources. This topic has resulted in multiple papers discussing the ethics of repeat valve replacement in an injection drug user with opinion still rather split.\textsuperscript{74,75,78} There seems to be cautiously increasing willingness to operate on this population over time and many note that they
would not feel comfortable flatly denying surgical or repeat surgical intervention if the patient did not have full access to drug treatment programs 75,78,79.

**Platelet’s Multi-Functional Role in Infection**

Given the difficulties of endocarditis diagnosis and treatment detailed above, investigators are interested in better understanding the basic pathophysiology of the disease in the hopes that this will allow new insights and improved clinical decision making. A key area of study is the role of platelets. Platelets are anucleate, disc-shaped cell fragments of 1-3 micrometers in diameter. An average adult has 150,000 to 450,000 platelets per microliter of blood, each lasting about 7-10 days in blood circulation. These multitudes of corpuscles are formed from large cells called megakaryocytes. Megakaryocytes initially develop in the bone marrow from a committed myeloid progenitor cell. They make up only about 0.01% of nucleated bone marrow cells though their numbers can be up-regulated 80. Thrombopoietin is the major regulator of megakaryopoiesis but IL-1, IL-6, and IL-11 are also stimulants 81. These cells are quite large in diameter and range from 50-100 micrometers with redundant, invaginated cell membranes 80. Given this large size, megakaryocytes do the bulk of their work releasing platelets directly into sinusoids of the bone marrow or trapped within the pulmonary capillary system 82. They form long, thin processes called proplatelets from which platelets bud; a megakaryocyte essentially fractures itself into hundreds of platelets 80. Prior to being shed, megakaryocytes actively pack the platelets with the molecules and machinery they will need to fulfill their function but will be unable to make themselves without a nucleus. This includes mRNA, protein translational machinery, and alpha
granules which are packets containing platelet factor 4, beta-thromboglobulin, fibrinogen, metalloproteases, and key platelet surface proteins\textsuperscript{82}. 

In a form of biological irony, platelets are the most simplistic of the blood corpuscles but play key roles in the complex hemostatic functions of vascular repair, inflammation, and host defense. They are most known for their role in clotting and repair of damage to the vascular system. When vessel injury occurs, collagen, von Willebrand factor (vWF), and tissue factor are exposed. While tissue factor begins the clotting cascade, the platelet receptors GpIa/IIa and α2β1 bind collagen, Gp1b/9/10 binds vWF, and Gp2b3a receptors bind fibrinogen which all serve to effectively bind the platelet in place. This binding frequently also serves to ‘activate’ the platelet resulting in a sudden and irreversible conformational change with release of various molecules. These secreted products include vascular endothelial growth factor, basic fibroblast growth factor, epidermal growth factor, and many other proteins and orchestrators key to vascular repair\textsuperscript{83}. They also release mediators such as thromboxane A2, ADP, and serotonin that activate and attract additional platelets to the ever growing platelet plug. Meanwhile, the coagulation cascade has been busy forming thrombin which cross links and further activates platelets. Thus the result of any endothelial injury is rapid aggregation and activation of platelets which serve not only to plug the damage but to actively aid in repair.

Platelets also have a strong antibacterial effect. This makes logical sense given that vasculature in the skin or abdomen that needs repairing would potentially be exposed to an influx of bacteria. Platelets exert their antimicrobial effects through their secretions and their complex interactions with antibodies and complement. Secretory molecules
within platelets are varied and include agents such as platelet factor 4 (a chemokine)\textsuperscript{84}, connective tissue activating peptides (aid in inflammation and wound repair)\textsuperscript{85}, chemokine ligand 5 (a chemokine)\textsuperscript{86}, and fibrinopeptides (cleavage products of fibrinogen which are inflammatory)\textsuperscript{87}. These are released when bacteria bind to platelets; the effects of the secretions vary between different organisms and strains. For example, some S. aureus strains are more resistant to the microbicidal proteins of platelets because they have altered their membrane fluidity, membrane potential, or acquired a plasmid-encoded efflux pump. The ability to withstand platelet microbicidal proteins is essential for endocarditis-causing organisms. These strains will be able to circulate longer in the bloodstream than those that are more susceptible to microbicidal peptides and persist within vegetations\textsuperscript{88-90}. This has been confirmed in patients when Viridans streptococci from patients with IE were compared to oral Viridans streptococci and strains from patients with infections other than IE. The IE strains were significantly more resistant to the activity of platelet microbicidal proteins\textsuperscript{91}. Further study suggests that platelet microbicidal proteins are not only helpful in and of themselves, but may exert synergistic effects with antibiotics\textsuperscript{92}.

Staphylococcus aureus interacts in many ways with platelets and its interactions are some of the best characterized. Alpha toxin is a potent pore-inducing hemolysin secreted by many S aureus strains but it also is a potent platelet aggregator at sublytic concentrations\textsuperscript{93}. It may seem unclear why an organism would want the ability to aggregate platelets given their antibacterial effects cited above. Benefits include potential plugging of the vasculature to prevent the arrival of additional immune effector cells. It also can result in the organism being hidden within a group of platelets which makes it
harder for other, more effective cells such as neutrophils to reach it. While platelets have been known to engulf bacteria, it is highly likely that S. aureus continues to survive within the platelet where they are even further hidden from more effective immunologic means of clearance. It is worth noting for the condition of endocarditis, however, that S. aureus strains with high levels of alpha toxin secretion were LESS capable of causing endocarditis; this is presumed to be due to the fact that high alpha toxin levels rupture the platelet and release its microbicidal proteins at extremely high concentrations.

More specific aggregators of platelets that are harbored by S. aureus are clumping factor A and B (clfA, clfB). ClfA has a fibrinogen binding domain projected from the cell surface with the fibrinogen then acting as a bridge to the platelet’s GPIIb/IIIa receptor. A threshold number of clfA molecules must be bound before platelet aggregation occurs, but once it occurs then aggregation is rapid. A second signal is needed to activate a platelet: a clfA-specific immunoglobulin bound to the platelet surface must bind the clumping factor. These immunoglobulins are thought to be present for most of the endocarditis-causing organisms as they are most frequently commensals. This mechanism results in rapid activation. Clumping factor A allows S. aureus-mediated platelet aggregation under high shear forces such as those found in endocarditis and in fact seems to result in even more aggregation under high shear conditions than low shear.

Clumping factors are most effective in the active growth stage of S. aureus infection while fibronectin binding proteins A and B (FnBPA and FnBPB) take on a larger role in the resting stage. This is thought to be due to how genes are regulated and transcribed during different growth phases of the organism. FnBPA works in a manner.
similar to clfA: it also is capable of activation using fibronectin as a bridge between the binding protein on the bacterium and the GpIIb/IIIa integrin of the platelet. It too will then still require a second IgG mediated signal for activation. Fibronectin binding protein B is less effective than FnBPA in that it can adhere to platelets but is far less capable of aggregating them\textsuperscript{97}.

Additional proteins of S. aureus have smaller roles in aggregating and activating platelets. A large collection of these more minor players are members of the large protein family known as the serine-aspartate repeat (Sdr) protein family, which closely resemble the structure of clumping factor proteins. SdrC, SdrD, and SdrE have all been identified. SdrE is a protein with multiple effects. It binds a key regulator of the complement pathway called Factor H so that the complement cascade and its bactericidal effects are hampered\textsuperscript{98}. It is also proven to be capable of causing platelet aggregation\textsuperscript{99}. Protein A is another low level aggregator. Protein A is a cell wall protein that binds the Fc region of IgG in addition to binding vWF. The vWF serves as a bridge between the bacterium and platelet. Protein A can also allow direct binding of platelets and S. aureus by binding the gC1qR found on many cells including platelets\textsuperscript{100}. Finally, the surface protein SraP (serine-rich adhesion for platelets), is another receptor capable of directly binding platelets to S aureus\textsuperscript{101}.

Streptococci also have multiple, diverse, interactions with platelets. S. sanguis uses a protein called Platelet aggregation associated protein (PAAP) which resembles collagen and to which the platelet is ‘tricked’ into binding\textsuperscript{102}. Group A Streptococci’s M protein can mediate platelet aggregation through an antibody and complement dependent manner. S. Gordonii has an adhesion called GspB that allows direct binding to human
platelets through the GPIbα platelet ligand\textsuperscript{103,104}; the S aureus protein SraP above is the Staphylococcal homolog of this gene\textsuperscript{101}.

**Platelet Importance in Endocarditis Pathophysiology**

Strains of bacteria adept at causing endocarditis rely heavily on their abilities to activate and aggregate platelets. Strains of S. aureus that lack the ability to directly bind platelets are significantly less likely to cause endocarditis in rabbit models\textsuperscript{105}. Even when endocarditis does develop, the vegetations contain significantly less bacteria\textsuperscript{105}. A study of 2 strains of Streptococcus sanguis in which one had the ability to aggregate platelets and one did not found that while both strains could result in rat endocarditis, the aggregating strain had higher bacterial concentrations within vegetations\textsuperscript{106}. Streptococcus gordonii strains that lack GspB seem less able to cause endocarditis and then form smaller vegetations containing fewer bacteria when they do manage to establish endocarditis\textsuperscript{107}. When PAAP is neutralized, experimental endocarditis with streptococcal species runs a milder course with smaller vegetations\textsuperscript{108}.

The reliance of endocarditis bacteria on their platelet interactions results from the basic fact that formation of a platelet and fibrin vegetation affects all aspects of endocarditis. This is true in the superficial, clinical sense in that we use detection of a vegetation on echocardiography to help distinguish endocarditis from other infectious entities such as simple bacteremia\textsuperscript{22}. It is also the embolization of pieces of this vegetation to skin, arterioles, and other organs that result in many of the clinical characteristics and morbidity of the disease. This vegetation is also intrinsically tied to the deepest, most basic pathophysiology of the disease. Leukocytes such as neutrophils are not present in vegetations and bacterial-specific host antibodies cannot penetrate
them. This means that bacteria are insulated within the vegetation from the immune system mechanisms most adept to destroy them.

The vegetation is also thought to affect antibiotic efficacy. To treat even a simple blood stream infection, one must use an antibiotic capable of achieving cidal concentrations in the serum long enough to be effective. Many antibiotics are incapable of this. In endocarditis, the antibiotic must now also be able to diffuse in high concentrations through layers of platelet and fibrin with platelets also incorporated into the final layers of bacterial biofilm. One of the theories offered early on to explain why penicillin was capable of curing endocarditis when sulfonamides were not, was the fact that sulfonamides were found to be unable to diffuse through fibrin. Such hypotheses have resulted in multiple studies testing the theory that certain antibiotics do not penetrate well into vegetations and thus will have decreased endocarditis cure rates. Studies which were done on homogenized vegetations of rabbit model endocarditis found that concentrations in the vegetation resembled those in the serum and cast doubt on this theory. It was noted, however, that since the vegetations were homogenized prior to analysis that potentially unequal antibiotic diffusion throughout the vegetations was not able to be tested and may prove important. As technology advanced, studies were done examining how different antibiotics diffused into different parts of the vegetation. It was found that different antibiotics do indeed penetrate differently. Teicoplanin barely diffuses at all into a vegetation and is mostly active on the surface. Other drugs such as ceftriaxone and penicillin diffuse to the center but with a much lower concentration than on the periphery. Finally other drugs like aminoglycosides seem capable of full penetration.
While the more superficial effect of many antibiotics on vegetations partly explains the prolonged duration of therapy needed to treat endocarditis, another reason is that the vegetation allows bacterial colonies to enter a resting state. Those bacterial colonies deep within the center of the vegetation become less metabolically active. Cidal antibiotics such as the benzopenicillins are only able to exert their effects while bacteria are actively dividing as they disrupt and destroy cell wall assembly; inert bacteria are resistant to the usual penicillins effects. Additionally, the nutrition deficiencies experienced by these deeper organisms that have been present longest in the vegetation develop morphological changes in cell wall thickness and increase their excretion of polysaccharides to form biofilms which further alter antibiotic susceptibility.

Ultimately we see that platelets play both a facilitating and antagonizing role in endocarditis making their overall net balance of harm or benefit difficult to discern. On one hand, platelets strongly increase vegetation formation and all the benefits conferred to the bacteria from this formation. On the other hand, platelets play a strong role in our innate immune system to destroy pathogens. A clinical study to help illustrate the net gain or harm of platelets was a study performed by Sullam et. al. in 1993. Anti-platelet sera were developed in sheep and administered to study rabbits prior to inducing endocarditis. Study rabbits thus contracted endocarditis with S. sanguis while thrombocytopenic; controls had normal platelets counts. Ultimately the vegetations formed in the thrombocytopenic rabbits were smaller as would be expected. However, within this smaller vegetation the absolute number and density of bacteria was greatly
increased. The net effect of this change on mortality and embolic rates was unfortunately not fully elucidated as all rabbits were sacrificed at 72 hours.

In an attempt to better clinically understand the net balance of platelets in endocarditis as well as provide potential therapeutic benefit, many studies have explored the use of antiplatelet agents in endocarditis. Aspirin is an acetylated salicylate that inhibits cyclooxygenase-1 and-2 to decrease prostaglandin production by cells and disarms platelets producing a sort of functional thrombocytopenia\textsuperscript{119}. Multiple studies have shown that aspirin decreases vegetation size and bacterial density in animal models, generally in a dose-dependent manner\textsuperscript{120-122}. A potential explanation for why both vegetation size AND bacterial density are decreased by aspirin despite the increased density found in the thrombocytopenic rabbits above, is the fact that aspirin has additional direct antibacterial effects in addition to its . It appears that the salicylic component of aspirin is the key factor; both aspirin and salicylic acid can decrease binding to valvular epithelium and lessen S aureus platelet binding but salicylic acid has greater effect than aspirin\textsuperscript{123}. The salicylic acid of aspirin downregulates alpha hemolysin secretion and FnBPA in S. aureus\textsuperscript{124}. Salicylic acid in fact affects multiple genes in S aureus\textsuperscript{125,126} including some effects which may counterbalance their antibacterial effects thus far discussed. For example, salicylic acid mediated decrease in one of the capsular polysaccharide coats actually seems to allow increased invasion of epithelial cells by S aureus\textsuperscript{126}. Also the presence of salicylate may ultimately result in a combination of changes to metabolic pathways that result in decreased efficacy of antibiotics\textsuperscript{125}.

Given the mixed basic science and animal model evidence for aspirin and salicylic acid in S aureus infections and endocarditis, investigators have turned their
attention to clinical studies. In a randomized, double-blind, placebo-controlled trial of aspirin treatment at 325 mg/day for 4 weeks in patients diagnosed with IE it was tested if aspirin treatment decreased embolic events\textsuperscript{127}. 60 patients were in the treatment arm with 55 in the placebo arm. There was no significant different in the number of emboli and the aspirin arm trended toward increased bleeding risk. The question was further examined to see if aspirin had higher effect when taken PRIOR to endocarditis in a retrospective review of 241 patients, 75 of whom were on chronic antiplatelet therapy prior to developing endocarditis. Those on chronic therapy had lower all-cause mortality at 90 days but no difference in embolic rates\textsuperscript{128}. This is in contrast to a retrospective study from 1980-1998 of 600 IE patients with 147 on antiplatelet therapy at least 6 months prior to endocarditis diagnosis; in this study the risk of symptomatic emboli was reduced in those on antiplatelet therapy even when additional confounders were accounted for\textsuperscript{129}. This same group, however, then went back and assessed those excluded from the randomized clinical trial above due to long term aspirin use and compared them to the placebo arm (84 treatment to 55 placebo). The aspirin did not have an impact on the risk of embolic events but did indicate that the aspirin use may be associated with excess bleeding\textsuperscript{130}. Finally, in 2011, 283 patients with IE found that there was no significant difference in the propensity-adjusted rate of 6-month mortality nor symptomatic embolic events in those who were on chronic antiplatelet therapy (aspirin dipyridamole, clopidogrel, or ticlopidine\textsuperscript{131}). This puts the score at 2 trials, one of very high quality, showing no benefit for embolic disease but increased bleeding risk, 1 propensity study showing no benefit, and 1 retrospective review showing 90 day mortality benefit.
It is worth noting that aspirin’s direct antimicrobial effects may be somewhat specific to S. aureus and thus may be particularly effective only in S. aureus endocarditis cases. Evidence for such an argument comes from Sedlacek et. al. who found that aspirin treatment was associated with fewer S. aureus tunneled catheter bloodstream infections in hemodialysis patients; this protective effect was not seen for blood stream infections with other organisms\textsuperscript{132}. A similar study where all blood stream infections were examined did not find a difference\textsuperscript{133}. Recent work by the International Collaboration on Endocarditis who performed a multinational prospective study on IE found on an analysis of 670 patients that recent aspirin usage was ‘associated with a significantly decreased overall rate of acute valve replacement surgery (OR 0.58 [95\% CI 0.35-0.97]; p<0.04), particularly where valvular regurgitation, congestive heart failure or periannular abscess was the indication for such surgery (OR 0.46 [0.25-0.86]; p<0.02)\textsuperscript{134}.’ Thus further S. aureus specific IE evaluation with aspirin may be warranted.

**Platelet Count and Mean Platelet Volume as Markers of Disease Including Endocarditis**

While the benefit of treating platelets involved in endocarditis with agents such as aspirin requires further investigation, monitoring of platelets still is useful in a wide variety of infectious circumstances. Platelets have long been observed to be elevated in inflammation and decline in critical illness such as sepsis. Furthermore, thrombocytopenia has been found to be associated with worse outcomes in critically ill patients\textsuperscript{135-137}. In a study of critically ill patients admitted to intensive care units, platelet levels were trended between those who survived and those who succumbed to illness\textsuperscript{138}. In both groups the platelets downtrended in days 1-4 and then began to rise to normal
levels as marrow production increased. The surviving group continued to have rising platelet levels with thrombocytosis starting day 9. Conversely, those who died had a leveling off of platelets at normal levels. Those with thrombocytopenia at any point had higher mortality but thrombocytopenia at day 14 was an even greater predictor of mortality. This trend holds true in those specifically with critical illness due to sepsis. Ultimately the importance of platelet count as a marker for disease severity is underscored by its inclusion in both the sepsis-related organ failure assessment score (SOFA) and multiple organ dysfunction score (MODS).

The mechanisms of thrombocytopenia in sepsis and critical illness are poorly understood. Sepsis is a complex condition of activated monocytes and neutrophils triggered by recognition of conserved bacterial proteins. The resulting inflammatory cascade of cytokines and molecular mediators results in increasing numbers of activated immune and endothelial cells in a feedback loop. These same chemokines also result in a prothrombotic state. This is due to the physiology of platelet production and function. It is thought that the increase in low specificity antiplatelet antibodies that rise during this inflammatory cascade play a role in sepsis related thrombocytopenia. Possible interactions with drugs including heparin given to hospitalized patients may also result in some level of drug induced thrombocytopenia. Finally, platelets will be increasingly engaged in interactions with vascular beds and other cells preventing their ongoing circulation amidst the chaotic milieu of sepsis. Many can become entrapped in fibrin clots evolving in the prothrombotic state of sepsis. When the inflammatory cascade tips beyond the control of regulatory feedback loops the condition of disseminated intravascular coagulation can occur: a systemic depletion of clotting factors and platelets.
resulting in hemorrhage and tissue ischemia from diffuse intravascular microthrombosis\textsuperscript{144}.

In an attempt to better harness the diagnostic and prognostic capabilities of platelet measures, investigators have recently turned to the mean platelet volume (MPV). Mean platelet volume is an automated blood measure included as a part of routine blood chemistries. Larger volume platelets, measured in femtoliters (fL), contain more alpha granules, produce larger amounts of prothrombotic factors, aggregate more rapidly, and have higher numbers of adhesion molecules\textsuperscript{145}. It seems that larger platelets are released during times of physiologic stress and inflammation with additional changes in volume occurring when platelets undergo activation\textsuperscript{146}. The release of larger platelets from megakaryocytes in fact may be the tradeoff for lower numbers of platelets seen in severe illness. Furthermore, MPV seems to rise only in cases of acute platelet destruction\textsuperscript{146}. Thus MPV has been investigated in a rising number of studies as a more sensitive marker of inflammation and infection than the platelet count itself.

MPV has been found to be elevated in a broad range of conditions including autoimmune disease\textsuperscript{147}, diabetes\textsuperscript{148}, and a host of cardiovascular conditions including acute myocardial infraction and ischemic stroke\textsuperscript{146}. Furthermore, high MPV appears to correlate with worse outcomes of myocardial and cerebral infarction and could possibly even be used to predict subsequent events\textsuperscript{146}. Rise in MPV has also been found to be significantly associated with severe sepsis and septic shock as well as to be associated with increased mortality\textsuperscript{149-151}.

In regards to the relationship between MPV and endocarditis, the results of MESH terms [mean platelet volume] and [endocarditis] in PubMed returns 7 results: 3
relevant studies, 3 published responses to the articles, and one study unrelated to endocarditis. Review of the citations of these studies and letters revealed 2 additional relevant studies which were assessed. The first study is a small retrospective study performed by Gunebakmaz et al.\(^\text{152}\) (Table I). In this study, 40 patients with a mix of prosthetic valve and native valve endocarditis were retrospectively evaluated to assess the associations between IE and MPV. They found that high MPV levels were associated with more complications, embolism, and death; limiting the value of this study was the fact that only univariate comparisons were performed and few additional confounders were reported. Icli et al. assessed the overall trends of MPV in the course of endocarditis.\(^\text{153}\) In this study 29 patients with left sided endocarditis were followed for 2 weeks after the start of specific antibiotic therapy. It was found that MPV values were significantly higher in the IE group as compared to 29 controls and that the levels steadily declined with treatment: 9.86 ± 1.1 vs. 8.0 ± 1.0 fl, respectively; p < 0.01. Cho et al. followed on this work with serial measurements of MPV in 22 IE patients as compared to controls with similar findings to Icli.\(^\text{154}\) They also found that the mpv/platelet count ratio was significantly decreased in the platelet group. Ileri et al. examined 76 patients with definite IE and 34 age/gender matched controls to assess MPV association with embolic events.\(^\text{155}\) They found that MPV at hospital admission was significantly higher in those patients who suffered major embolic events that those who did not and controls (10.62 ± 1.13 vs. 9.25 ± 0.97 and 8.93 ± 0.82 fL, p < 0.001, respectively); they also found that mean platelet width was significantly higher than those without emboli and controls. As a notable confounder, those patients with embolic events were noted to have larger vegetations. Logistic regression was not used as an attempt to control for confounders.
The most statistically robust and comprehensive work was performed by Tok et al. where 108 patients with definite IE per Duke criteria were retrospectively analyzed\textsuperscript{156}. MPV was assessed at time of admission with cases grouped as MPV $\leq 8.6$ fL on admission and those with an MPV $>8.6$ fL. Univariate analysis showed those in the higher MPV group had significantly more ESRD, S. aureus infection, embolic events (32 vs 11\% 0.010) and in hospital mortality (28 vs 7\% p-0.005). These p values were unadjusted for multiple comparison testing. On multivariable Cox regression analysis however, MPV remained an independent predictor of a composite endpoint of embolic events and in hospital mortality with a HR of 1.69 (95\% CI of 1.54-1.86, p<0.0001)
Table I: A summary of the studies assessing associations between IE and MPV

<table>
<thead>
<tr>
<th>Author</th>
<th>Study</th>
<th>Population</th>
<th>Statistics</th>
<th>Conclusions</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunebakmaz et al.</td>
<td>Published 2010</td>
<td>Single center, Retrospective</td>
<td>T-test, ANOVA, chi square, paired T-test</td>
<td>-MPV sig. decreased from admission to discharge</td>
<td>-No control for confounders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 NV and 13 prosthetic</td>
<td></td>
<td>-MPV sig. higher in those with embolic complications and death</td>
<td></td>
</tr>
<tr>
<td>Icli et al.</td>
<td>Published 2013</td>
<td>Single center, prospective</td>
<td>T-test, Mann-Whitney U, ANCOVA to assess glucose impact, Chi square, paired T-Test</td>
<td>-MPV was significantly higher among patients with IE compared to controls</td>
<td>-Unclear how controls selected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29 consecutive left-sided IE including prosthetic valves with 29 controls</td>
<td></td>
<td>-MPV significantly decreased with IE treatment</td>
<td>-No control for confounders</td>
</tr>
<tr>
<td>Cho et al.</td>
<td>Published</td>
<td>Single center, prospective</td>
<td>T-test, Pearson’s correlation,</td>
<td>-Average MPV was significantly higher in case vs control</td>
<td>-univariate analyses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 IE patients with serial MPV measurements; 248 measures total</td>
<td></td>
<td>-mpv/plt ratio was sig increased in case vs control</td>
<td>-small sample size</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-MPV and CRP correlation of 0.21</td>
<td>-limited methods and results data reported</td>
</tr>
<tr>
<td>Ileri et. al.</td>
<td>Published 2015</td>
<td>Single center, prospective</td>
<td>T-test, Mann-Whitney U, Chi square</td>
<td>-Higher MPV at admission significantly associated with major embolic events</td>
<td>- more emboli had larger vegetations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76 definite IE with 34 age/gender matched controls</td>
<td></td>
<td></td>
<td>-no control for confounders</td>
</tr>
<tr>
<td>Tok et. al.</td>
<td>Published 2015</td>
<td>2 center, retrospective</td>
<td>T-test, Mann Whitney U, Chi square, Variables with p value &lt;0.1 in Cox regression analysis used in multivariable Cox regression model. Log-rank test</td>
<td>In multivariate analysis, MPV remained significantly associated with the combined outcome of embolic event and in-hospital mortality</td>
<td>-composite outcome used</td>
</tr>
</tbody>
</table>
Of the literature to date, only a single study attempted to account for confounders in their assessment of MPV associations. This may in part be due to the challenge of considering the sheer number of potential confounders which exist for both endocarditis outcomes and MPV. As noted above, multiple disease conditions have been found to be associated with MPV. Attempting to control for confounding conditions such as malignancy, rheumatic diseases, hypertension\textsuperscript{157} and type 2 diabetes\textsuperscript{158} would, however, improve validity. Further chronic conditions which have been shown to have association with increased MPV include aortic stenosis\textsuperscript{157}, mitral stenosis\textsuperscript{159}, atrial fibrillation\textsuperscript{160} and obesity\textsuperscript{161}. Presence of anti-platelet medication could be considered as a potential confounder, but it is noted that aspirin was shown not to have large effect on MPV\textsuperscript{162}. Measures of general inflammatory markers should also be considered for inclusion in a regression equation in order to show that MPV has independent value even in the presence of these other markers. Examples of these factors include fairly commonplace markers such as procalcitonin\textsuperscript{163} and c-reactive protein\textsuperscript{164}; some highly specialized markers such as cystatin c and matrix metalloproteinase-9 have also been found to be associated with infective endocarditis but would not be available to retrospective studies\textsuperscript{165}. Finally, disease related confounders on morbidity and mortality outcomes must be taken into account including embolic disease, valve involved, presence of heart failure, and whether the causative agent is a highly virulent organism such as S. aureus or fungus\textsuperscript{166,167}. Analysis of the above factors should be performed thoughtfully as correlations between these lab values, co-morbidities, and disease characteristics are likely.
Ultimately MPV holds promise as a potential diagnostic and prognostic aid in a number of conditions including endocarditis. Gaps in the literature regarding association of MPV and endocarditis include the lack of studies accounting for covariates and lack of any study assessing MPV in IDU-endocarditis. IDU-endocarditis may have a separate and unique association with MPV as IDU-IE may have additional platelet dysfunction resulting from particulate matter and drug induced thrombocytopenias.

Drugs are capable of causing platelet anomalies ranging from drug-induced immune thrombocytopenia (DITP) to thrombotic thrombocytopenic purpura syndrome (TTP). A systematic review of the literature performed by Al-nouri et al. found 78 different drugs that were suspected of causing thrombotic microangiopathy with 22 of them having enough evidence to suggest a causal relationship; of note cocaine was included on this list\textsuperscript{168}. Drugs can also cause basic thrombocytopenia through immune mediated and immune independent disease mechanisms\textsuperscript{169}. Generally, the nonimmune effects are mediated through direct marrow effects and are seen in chemotherapy agents. Heparin has a unique mechanism of action whereby it binds to platelet factor 4 to produce an immune complex which then activates platelets through Fc receptors\textsuperscript{169,170}. Finally, a small percentage of patients taking a drug can develop autoimmune antibodies or drug-induced immune complexes which result in platelet destruction of varying degrees; approximately 10 persons per million are affected by DITP annually\textsuperscript{171}. Six different mechanisms of DITP have been discovered and more than 10 drugs are capable of causing such reactions\textsuperscript{169}.

Wake Forest Baptist Hospital is a tertiary care hospital located in the Northwest corner of N. Carolina. The catchment area includes western parts of N. Carolina, southern
Virginia, and eastern aspects of West Virginia. We have observed rising numbers of IDU-IE cases. What is more, many report injection of the drug oxymorphone. This is a morphine derivative made available in oral formulation in 2006 by Endo pharmaceuticals\textsuperscript{172,173}. It is notable for its unique association with a microangiopathic hemolytic anemia (MAHA)\textsuperscript{174,175} and its notorious association with an HIV outbreak of Scott county Indiana\textsuperscript{176}. The Wake Forest population of oxymorphone abusers could provide novel insights about the role of platelets and MPV in endocarditis given that this group has a high proportion of patients presenting with a MAHA resembling TTP. While endocarditis itself can mimic this disease\textsuperscript{177}, it seems that it may be the drug itself resulting in this effect rather than the infection. This would mean that the platelet destruction and enlarged MPV of inflammation could potentially have started even prior to the endocarditis and potentially result in even more profound elevations in MPV at admission. I seek to assess if the previously observed association between MPV and IE hold true in the IDU-IE population and if, specifically, this association is enhanced or attenuated by specific use of the drug oxymorphone.
CHAPTER 2: Measurement of Association Between Mean Platelet Volume and Septic Emboli in Endocarditis

Introduction

Platelets are small, anucleate cell fragments which play a role in everything from hemostasis to inflammation and host defense. Platelets secrete chemokines and microbicidal proteins while also engaging in complex interactions with antibodies, complement, and bacteria themselves\(^{96,98}\). Larger volume platelets contain more prothrombotic factors, alpha granules, adhesion molecules and aggregate more rapidly; platelets gain additional volume when activated\(^{145}\). For these reasons mean platelet volume (MPV) has been increasingly evaluated as a potential marker and predictor of infection and inflammation. MPV has been found to have significant association with diseases ranging from diabetes to autoimmune diseases and high MPV has been found to correlate with worse outcomes of myocardial and cerebral infarction\(^{146-148}\). A handful of studies have attempted to evaluate associations between MPV and endocarditis (IE) outcomes\(^{152-156}\) with interesting findings of an association between MPV and major embolic events alone\(^{155}\) and with a combined outcome of embolic event and in-hospital mortality\(^{156}\). Unfortunately, these previous studies have suffered from small sample sizes and incomplete confounder analysis. Furthermore, no study has assessed MPV in the setting of injection drug use associated infective endocarditis (IDU-IE). Multiple drugs are known to have effect on platelet number and behavior and injection drug use may thus impact any outcomes associated with MPV\(^{168,169}\). In this cross-sectional, observational study we present the largest assessment of association between MPV and IE embolic phenomena as well as the first assessment of MPV with IDU-IE.
Methods
Patient Population

Patients >=18 years old admitted to Wake Forest Baptist Medical Center (WFBMC) from 1/1/2004-12/30/2016 with IE were identified by searching all encounters I33.9, A32.8, A54.83, B37.6 and I38. That query resulted in a dataset of 2370 unique medical records for ICD-9 and 33 for ICD-10. Individuals that had a simultaneous ICD-9 code of 996.0, ‘mechanical complication of cardiac device, implant and graft,’ were removed in an effort to exclude device-related endocarditis. The 1400 remaining charts were reviewed by a single author (EB) to screen for exclusion criteria: previous endocarditis, onset of infectious symptoms >72 hours after hospital admission, marantic endocarditis, and failure to meet at least ‘possible’ endocarditis by the modified Duke criteria\textsuperscript{22} (Table II and Table III)
## Table II: Major and Minor Modified Duke Criteria

| **Major Criterion:** Blood Culture | **1)** 2 separate blood cultures positive for Viridans streptococcus, S. bovis, HACEK group, S. aureus, or community-acquired enterococci  
|                                 | **2)** At least 2 positive cultures of blood samples drawn >12 hours apart with the above organism  
|                                 | **3)** All of 3 OR a majority of ≥4 separate blood cultures with the above organisms and with the first and last sample drawn at least 1 hour apart  
|                                 | **4)** Coxiella burnetti antiphase 1 IgG antibody titer >1:800 OR single positive blood culture for this organism  
| **Major Criterion:** Echocardiographic | **1)** Oscillating intracardiac mass on valve or supporting structures, in path of regurgitant jets, or on implanted material in absence of alternative anatomic explanation  
|                                 | **2)** Echocardiogram positive for abscess  
|                                 | **3)** New Partial dehiscence of prosthetic valve  
|                                 | **4)** New valvular regurgitation  
| **Minor:** Predisposition | Predisposing heart condition including bicuspid aortic valve or injection drug use  
| **Minor:** Fever | Temperature >38 degrees Celsius, >100.4 degrees Fahrenheit  
| **Minor:** Vascular | Major arterial emboli, septic pulmonary infaracts, mycotic aneurysm, intracranial hemorrhage**, conjunctival hemorrhages, Janeway’s lesions  
| **Minor:** Immunologic | Glomerulonephritis, Osler’s nodes, roth’s spots, rheumatoid factor  
| **Minor:** Microbiologic | Positive blood culture that does not meet major criterion  

** For purposes of this study and consistent with the literature¹⁷⁸,¹⁷⁹, both hemorrhagic and ischemic cerebrovascular events were considered as fulfilling this criterion so long as the radiographic reading stated the lesions were likely embolic in nature and the clinical care team considered them as related to the endocarditis
Table III: Definite Versus Possible Endocarditis Diagnosis By Modified Duke Criteria

<table>
<thead>
<tr>
<th>Definite</th>
<th>Microorganisms demonstrated by culture or histologic exam from a vegetation, a vegetation that has embolized, or intracardiac abscess specimen</th>
</tr>
</thead>
</table>
| Definite | A. 2 Major Criteria  
B. 1 Major and 3 Minor Criteria  
C. 5 minor Criteria |
| Possible | A. 1 Major and 1 Minor Criteria  
B. 3 Minor Criteria |
| Rejected | A. Firm alternate diagnosis explaining evidence of IE  
B. Resolution of syndrome with antibiotic therapy for ≤4 days  
C. No pathologic evidence at surgery or autopsy with antibiotic therapy ≤4 days  
D. Does not meet criteria for possible endocarditis |

Data Variables and Management

Data from qualifying cases were collected using REDCap\textsuperscript{180}. Basic demographic data on race, sex, and age were extracted for all patients. Information pertaining to IDU included reported drug(s) of abuse and reported last injection. Key clinical features assessed were vegetations visualized by echocardiography, presence of embolic manifestations, metastatic foci of infection, and causative pathogen. A Charleson comorbidity score was calculated for all individuals as a measure of baseline medical status\textsuperscript{181,182} (Table IV). Additionally, the presence of atrial fibrillation and hypertension were assessed by reviewing the admission history and physicals and discharge summaries of all patients.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Status</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Uncomplicated</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>End-Organ Damage</td>
<td>+2</td>
</tr>
<tr>
<td>Liver Disease</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>Moderate to Severe</td>
<td>+3</td>
</tr>
<tr>
<td>Solid Tumor</td>
<td>Metastatic</td>
<td>+6</td>
</tr>
<tr>
<td></td>
<td>Non-Metastatic</td>
<td>+2</td>
</tr>
<tr>
<td>AIDS(^a)</td>
<td>Present</td>
<td>+6</td>
</tr>
<tr>
<td>Leukemia or Lymphoma</td>
<td>Present</td>
<td>+2</td>
</tr>
<tr>
<td>CKD(^b) (Moderate to Severe)</td>
<td>Present</td>
<td>+2</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>Present</td>
<td>+1</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>Present</td>
<td>+1</td>
</tr>
<tr>
<td>COPD(^c)</td>
<td>Present</td>
<td>+1</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>Present</td>
<td>+1</td>
</tr>
<tr>
<td>Stroke or TIA(^d)</td>
<td>Present</td>
<td>+1</td>
</tr>
<tr>
<td>Dementia</td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td></td>
<td>+2</td>
</tr>
<tr>
<td>Connective Tissue Disease</td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>Peptic Ulcer Disease</td>
<td></td>
<td>+1</td>
</tr>
</tbody>
</table>

\(^a\): Acquired Immunodeficiency Syndrome  
\(^b\): Chronic Kidney Disease  
\(^c\): Chronic Obstructive Pulmonary Disease  
\(^d\): Transient Ischemic Attack

Laboratory data of mean platelet volume measured in fL (MPV), white blood cell count measured in units x 10\(^9\)/L (WBC), albumin measured in g/dL, creatinine measured
in mg/dL, and platelet count measured in units x $10^3/\mu L$ were all recorded. These values were collected at the time of presentation to the emergency department at either WFBMC or an outside hospital (OSH). A large proportion of patients were transferred to WFBMC from outside facilities. Outside records which had been contemporaneously scanned into our medical record were reviewed for these patients. For those who originally presented to an OSH and were transferred to WFBMC within 24 hours of presentation, the first WFBMC values drawn were also recorded. Data from 2004 was excluded as no hospital had MPV on their complete blood counts prior to 2005. In order to minimize variation from different lab instrument measurements, OSH values were only used when no qualifying WFBMC measure was present i.e. those patients presenting to an outside facility who were not transferred to WFBMC within 24 hours.

**Definitions**

All cases of IE were defined using the Modified Duke Criteria\textsuperscript{183}. Patients with a previous history of endocarditis were excluded; all data is from primary episode endocarditis cases. An IDU-IE case was defined as IE in a patient who reported IDU within 3 months of admission. Drug use recorded as ‘recent’ was counted as fulfilling this time criterion. Cases were classified as oxymorphone exposed (OE) only if one of the reported drugs of injection was explicitly documented as oxymorphone. Cases where nonspecific terms such as ‘injected opioids ’ were used defaulted to the oxymorphone unexposed (OU) group.

A Large vegetation was defined as $\geq 10\text{mm}$ or being described as a ‘mass’ on the echocardiogram report. A case was considered to have a left side valve involved if a vegetation was seen on the valve in isolation or in combination with another valve.
Pulmonary emboli were defined as the presence on radiographic studies of scattered cavitary lesions consistent with septic emboli. Central Nervous System (CNS) emboli were defined as the presence on radiographic studies consistent with infarction in patients with neurologic symptoms.

**Statistical Analysis:**
Univariate analyses were assessed using Chi-Square, Fisher’s Exact Test, Mann-Whitney U, One-Way ANOVA, and Student’s T-test. To ensure data met required assumptions for logistic regression, independence was assessed for all categorical and continuous variables. A Pearson’s correlation coefficient (r) was calculated for all continuous variables while significance and strength of association between categorical variables were assessed using chi square with phi coefficient.

Logistic regression, log-binomial regression and poisson regression were utilized to assess the association between MPV and embolic phenomena while controlling for potential confounders. Four sequential models were assessed. The covariates controlled for were sex, Charleson comorbidity score, and age in model 1. Additional covariates controlled for in mode 2 were white blood cell count, total platelet count, albumin, creatinine, and whether the lab data were measured at an OSH, WFBMC, or WFBMC after transfer from OSH within 24 hours. Additional variables included in model 3 were atrial fibrillation and hypertension. Additional variables included in model 4 were Staphylococcus aureus as a causative organism, visualization of a large vegetation, visualization of a left-sided valve vegetation, and presence of IDU.

Overall model significance was assessed by Likelihood ratio and Wald test in logistic regression. Significance of individual parameter estimates was assessed; a 2-
sided p value <0.05 was considered significant. Odds ratio and relative risk with 95% confidence intervals were calculated. All analyses were performed using JmpPro 13 and SAS 9.4.

Results
Summary statistics:
310 cases of endocarditis cases meeting inclusion criteria were affirmed consisting of 93 IDU-IE cases and 217 non-IDU cases (Fig. 1). MPV was not tested at any facility prior to the year 2005 and 18 cases which occurred in 2004 were removed leaving 292 cases. Twenty-nine were further excluded from analysis due to a complete lack of laboratory data from the hospital where they originally presented and >24 hours before transfer to WFBMC. Finally, 26 were excluded from analysis for having a missing MPV value. This resulted in a total of 237 individuals analyzed which included 80 of IDU-IE cases (38 OU and 42 OE). The characteristics of the population, the admission laboratory values, and the clinical details of the endocarditis hospitalization are summarized in tables V, VI, and VII respectively.
Figure 1: Flow Diagram of Endocarditis Case Selection, Validation, And Exclusion

PULLED FROM ELECTRONIC DATABASE

Age >=18
ANY endocarditis ICD9 or ICD10 coded encounter
1/1/2004-12/31/2017
No endocarditis code prior to study period

2403

Removed
1003 with simultaneous ICD 996.0 code for cardiac device malfunction

1400

Removed
774 Not endocarditis
88 Previous episode of endocarditis or device present
9 Pediatrics

529

Removed
Hospital acquired and marantic endocarditis
‘Endocarditis’ did not meet Duke
Insufficient records to assess Duke Criteria

310

Removed
18 cases from 2004 when MPV test not used

292

Removed
29 without ANY original hospital data available AND > 24 hours to transfer

263

Removed
19 with lab data complete EXCEPT for an MPV measure
7 missing MPV and another lab value

237
### Table V: Demographic Characteristics of All Analyzed Endocarditis Cases

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>No Drug Use</th>
<th>Drug Use</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(% )</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>237</td>
<td>157 (66.2)</td>
<td>80 (33.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>46.9 ±17.5</td>
<td>54.3 ±15.9</td>
<td>32.3 ± 9.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Male</td>
<td>143 (60.3)</td>
<td>103 (64.6)</td>
<td>40 (50)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>94 (39.7)</td>
<td>54 (34.4)</td>
<td>40 (50)</td>
<td></td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>White</td>
<td>200 (84.4)</td>
<td>123 (78.3)</td>
<td>77 (96.3)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>32 (13.5)</td>
<td>30 (19.1)</td>
<td>2 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>5 (2.1)</td>
<td>4 (2.5)</td>
<td>1 (1.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Charleston</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median 1</td>
<td>1.1 ± 1.5</td>
<td>1.5 ± 1.7</td>
<td>0.375 ± 0.7</td>
<td>Median 0</td>
</tr>
<tr>
<td><strong>Prosthetic Valve</strong></td>
<td>13 (5.5)</td>
<td>0</td>
<td>13 (8.3%)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Atrial Fibrillation</strong></td>
<td>36 (15.2)</td>
<td>32 (20.4)</td>
<td>4 (5)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>98 (41.4%)</td>
<td>80 (51)</td>
<td>18 (22.5)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table VI: Admission Laboratory Values of All Analyzed Endocarditis Cases

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>No Drug Use</th>
<th>Drug Use</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(% )</td>
<td>N(%)</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OSH</td>
<td>53 (22.4)</td>
<td>41 (26.1)</td>
<td>12 (15)</td>
<td></td>
</tr>
<tr>
<td>WFBMC</td>
<td>91 (38.4)</td>
<td>78 (49.7)</td>
<td>13 (16.3)</td>
<td></td>
</tr>
<tr>
<td>Transfer</td>
<td>93 (39.2)</td>
<td>38 (24.2)</td>
<td>55 (68.8)</td>
<td></td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td>11.1</td>
<td>11</td>
<td>12.2</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>13.1 ±6.8</td>
<td>12.8 ±7</td>
<td>13.6±6.4</td>
<td></td>
</tr>
<tr>
<td><strong>Platelet</strong></td>
<td>154</td>
<td>187</td>
<td>98</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>185.1 ±136.3</td>
<td>201.3±135.3</td>
<td>153.3 ±133.5</td>
<td></td>
</tr>
<tr>
<td><strong>MPV</strong></td>
<td>8.9</td>
<td>8.6</td>
<td>9.4</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>9.1 ± 1.7</td>
<td>8.8 ±1.6</td>
<td>9.7 ± 1.8</td>
<td></td>
</tr>
<tr>
<td><strong>MPV ≥ Median</strong></td>
<td>126 (54.8)</td>
<td>71 (47)</td>
<td>55 (69.6)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>2.7</td>
<td>2.9</td>
<td>2.6</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2.8 ±0.7</td>
<td>2.9±0.7</td>
<td>2.6 ±0.6</td>
<td></td>
</tr>
<tr>
<td><strong>Creatinine</strong></td>
<td>1.1</td>
<td>1.2</td>
<td>0.9</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>1.8 ±2.1</td>
<td>1.9±2.1</td>
<td>1.7 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Table VII: Comparison of Endocarditis Clinical Characteristics Between Injection Drug Users and Non Injectors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td>Total N(%)</td>
<td>No Drug Use N(%)</td>
<td>Drug Use N(%)</td>
<td>P Value</td>
</tr>
<tr>
<td>Duke</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite</td>
<td>158 (66.7)</td>
<td>96 (61.2)</td>
<td>62 (77.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Possible</td>
<td>79 (33.3)</td>
<td>61 (38.8)</td>
<td>18 (22.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTE</td>
<td>231 (97.5)</td>
<td>153 (97.5)</td>
<td>78 (97.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>TEE</td>
<td>149 (62.9)</td>
<td>102 (65)</td>
<td>47 (58.8)</td>
<td>0.35</td>
</tr>
<tr>
<td>Large Vegetation</td>
<td>87 (36.7)</td>
<td>52 (33.1)</td>
<td>35 (43.8)</td>
<td>0.11</td>
</tr>
<tr>
<td>Valve Affected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricuspid</td>
<td>63 (26.6)</td>
<td>29 (18.5)</td>
<td>34 (42.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>3 (1.3)</td>
<td>2 (1.3)</td>
<td>1 (1.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mitral</td>
<td>82 (34.6)</td>
<td>64 (40.8)</td>
<td>18 (22.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Aortic</td>
<td>58 (24.5)</td>
<td>47 (29.9)</td>
<td>11 (13.8)</td>
<td>0.006</td>
</tr>
<tr>
<td>No Vegetation</td>
<td>41 (17.3)</td>
<td>23 (14.7)</td>
<td>18 (22.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>&gt;1 Valve</td>
<td>31 (13.1)</td>
<td>20 (12.7)</td>
<td>11 (13.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>LOS (days)</td>
<td>18.4 ±13.4</td>
<td>16.6 ±12</td>
<td>21.9 ±15.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Pulmonary Emboli</td>
<td>73 (30.8)</td>
<td>26 (16.6)</td>
<td>47 (58.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CNS Emboli</td>
<td>52 (21.9)</td>
<td>44 (28)</td>
<td>8 (10)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulmonary/CNS Emboli</td>
<td>115 (48.5)</td>
<td>65 (41.4)</td>
<td>50 (62.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Organism</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CoNS</td>
<td>12 (5)</td>
<td>12 (7.6)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>S aureus</td>
<td>125 (52.7)</td>
<td>64 (40.8)</td>
<td>61 (76.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>16 (6.8)</td>
<td>15 (9.6)</td>
<td>1 (1.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Viridans Streptococci</td>
<td>35 (14.8)</td>
<td>27 (17.2)</td>
<td>8 (10)</td>
<td>0.14</td>
</tr>
<tr>
<td>Culture Negative</td>
<td>19 (8)</td>
<td>15 (8.9)</td>
<td>5 (6.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>10 (4.2)</td>
<td>8 (5.1)</td>
<td>2 (2.5)</td>
<td>0.50</td>
</tr>
<tr>
<td>Other</td>
<td>20 (8.4)</td>
<td>17 (10.8)</td>
<td>3 (3.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>S aureus involved</td>
<td>127 (53.6)</td>
<td>65 (41.4)</td>
<td>61 (77.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>In-Hospital Mortality</td>
<td>46 (19.5)</td>
<td>40 (25.6)</td>
<td>6 (7.5)</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

TTE: transthoracic echocardiography was obtained; TEE: transesophageal echocardiography was obtained; LOS: length of stay in days; CNS: cerebral nervous system; CoNS: coagulase negative Staphylococcus species
Assessing relationships among variables:
The association of mean platelet volume with all other confounders was systematically assessed. The highest correlation was between mean platelet volume and total platelet count with a moderate, negative association of $r = -0.48$. No other categorical variables had a correlation greater than 20%. Among the nominal variables, multiple variables had significant associations as measured by chi square but none had phi values higher than 0.5 suggesting that variables were sufficiently independent for analysis (appendix I).

Logistic Regression:
Results of the logistic regression calculations are seen in Table VIII. MPV remained significantly, positively associated with septic emboli even in the fully adjusted model (OR 1.38; 95% CI: 1.1-1.7). Assuming stability in all other variables, the odds of an embolic event increase by 38% for each unit increase in MPV. Control for the variables WBC, total platelet count, creatinine, albumin, data source, hypertension, and atrial fibrillation all attenuated the relationship between MPV and embolic disease. However, inclusion of visualization of large vegetations or vegetations on left sided valves, S. aureus as a causative agent, and drug use status clarified and strengthened the association between MPV and embolic disease. This suggests that these latter variables explain large amounts of variability in the model and then allow weaker effects to show through more strongly. Given the frequency of the outcome, analyses were also evaluated with log-binomial and poisson regression adjusted for robust errors. These regressions confirm significance of the fully adjusted relationship with RR of 1.08 and 1.13 respectively (appendix II-IV).
Table VIII: Results of Multiple Logistic Regression Assessing Association Between Mean Platelet Volume And Embolic Phenomena of Endocarditis

<table>
<thead>
<tr>
<th>Model</th>
<th>LR</th>
<th>Model Significance</th>
<th>MPV  β</th>
<th>MPV SE</th>
<th>MPV Significance</th>
<th>OR  Lower</th>
<th>OR  Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>37.1</td>
<td>&lt;0.0001</td>
<td>0.3849</td>
<td>0.0922</td>
<td>&lt;0.0001</td>
<td>1.469</td>
<td>1.226</td>
</tr>
<tr>
<td>Model 2</td>
<td>46.6</td>
<td>&lt;0.0001</td>
<td>0.2930</td>
<td>0.1070</td>
<td>0.0062</td>
<td>1.340</td>
<td>1.087</td>
</tr>
<tr>
<td>Model 3</td>
<td>48.4</td>
<td>&lt;0.0001</td>
<td>0.2852</td>
<td>0.1073</td>
<td>0.0079</td>
<td>1.330</td>
<td>1.078</td>
</tr>
<tr>
<td>Model 4</td>
<td>61.4</td>
<td>&lt;0.0001</td>
<td>0.3199</td>
<td>0.1117</td>
<td>0.0042</td>
<td>1.377</td>
<td>1.106</td>
</tr>
</tbody>
</table>

Abbreviations: LR: likelihood ratio; MPV: mean platelet volume β: parameter estimate for mean platelet volume SE: standard error for the mean platelet volume parameter estimate; OR: odds ratio; CI: confidence interval

Model 1 variables: age, gender, age-unadjusted comorbidity score, mean platelet volume
Model 2 variables: model 1 and white blood cell count, platelet count, creatinine, albumin, and data source
Model 3 variables: model 2 and atrial fibrillation and hypertension
Model 4 variables: model 3 and staphylococcus aureus involvement, left sided valve vegetation seen, large vegetation seen, injection drug use status

Assessment of relationship between drug use status, MPV, and embolic disease

Drug-use status was not a significantly associated variable with embolic disease in the multiple logistic regression models despite its significant association in univariate modeling (p=0.003). This may be that the impact drug use has on embolic phenomena is explained by inclusion of other variables in the multiple regression model such as MPV. To assess this possibility, the relationships between MPV and drug use were assessed through one-way ANOVA and 2-sided t-tests. Additional investigations for interaction, moderation, and mediation were then conducted.

MPV was significantly associated with both drug use overall (drug use yes or no) (p=0.0004) and drug group (no drug use, OU, OE) (p=0.0005). MPV as a continuous variable was not significantly different between OE and OU groups when this population of 80 patients was assessed in isolation (p=0.12). However, when MPV was assessed as a dichotomous variable denoting MPV value as greater than or equal to the median of its
associated reference range or below its median, results changed. 80.2% of the OE group had an MPV greater than or equal to the reference range median versus only 59.5% of the OU group and 50.3% of the no drug user group (p=0.001). When compared directly, the OE group was significantly more likely to have an MPV value above the median than the OU group (p= 0.048).

Assessment for an interaction between MPV and drug use yielded an insignificant interaction term (p=0.81). Stratifying the analysis of MPV with embolic disease by drug use did not affect the OR of MPV: OR 1.449 [1.16-1.8] when no drug use and OR of 1.517 [1.21-2.05] when drug use was present.

**Discussion**

Mean platelet volume at admission remained significantly associated with embolic disease of endocarditis even when adjusted for multiple covariates with an OR of 1.38. Many confounders were controlled for including baseline comorbidity status, age and additional lab values of total wbc, total platelet count, creatinine, and albumin. Both creatinine and albumin have been found to have impact on endocarditis measurements of morbidity and mortality\textsuperscript{184,185}. We also adjusted for those variables which are known to influence embolic disease including size of vegetation, presence of atrial fibrillation, and side of the heart affected\textsuperscript{186}. S aureus involvement was controlled for as well given that S. aureus has unique platelet interactions as compared to the streptococci and has been singled out as a key factor in embolic disease\textsuperscript{96,101,102,106,178}. That MPV remained significant in the fully adjusted model suggests that platelet activation and aggregation play a significant role in endocarditis embolic disease beyond simply indicating the size of vegetation.
Our results are in concordance with the previous studies performed on this topic. Ileri et al prospectively studied 76 IE cases with 34 age and sex matched controls\textsuperscript{155}. They found that increased MPV at admission was significantly associated with increased embolic events. This study had a low event rate with only 13 embolic events and did not control for vegetation size in their model. Our study confirms these results in a more robust sample size and shows that this relationship holds true even when the size of the vegetation is taken into consideration. Tok et al. also found that MPV >8.6fL was significantly associated with the combined outcome of in-hospital mortality and embolism in a study of 108 definite IE patients as compared to those with a MPV <=8.6fL\textsuperscript{156}.

Injection drug use was not significantly associated with embolism in the fully adjusted model. It was, however, highly associated with MPV: those who injected drugs had a larger MPV on admission (p=0.004). In the drug user group alone, those exposed to oxymorphone, the OE-group, were significantly more likely to have an MPV above the median reference range than the OU-group (p=0.048). It may be that the constant interaction of platelets with injected particles and bacteria lead to a state of chronic activation and thus increased MPV even when not septic or acutely infected. Oxymorphone results in a microangiopathic hemolytic anemia of unclear pathogenesis\textsuperscript{175,187} which may further activate and aggregate platelets than other injected substances and account for the increased MPV in this group.

There is no evidence that mean platelet volume interacts with or moderates the relationship of MPV with endocarditis embolic disease. The interaction term, which can sometimes be difficult to power, was far from significant (p=0.8). If there was
moderation, the OR of MPV with embolic disease would significantly differ when the analysis was stratified by drug use; this was not the case.

**Limitations**

There are multiple limitations in our laboratory value assessments and control measures. Patients were transferred from more than 30 different facilities to Wake Forest and many had variable reference range scales for their laboratory values. We attempted to minimize this variability by using OSH data were when necessary and otherwise used lab values drawn at WFBMC. We further sought to minimize this factor by controlling for source in the regression models and confirming that the key measure of MPV was not associated with data source.

An additional limitation is that too few patients had sedimentation rates and c-reactive proteins drawn to allow for adjustment. These 2 inflammatory markers may well obscure or render irrelevant MPV’s association with embolic phenomena. However, the very fact that so few of these individuals had these tests performed indicates that these tests are not being routinely ordered and used. Thus any additional strength in association they may have over MPV is somewhat offset by convenience and ready availability of the MPV. We additionally lacked a robust enough population to look specifically at many other factors such as obesity, HDL, and individual conditions that have been shown to influence MPV. A more granular assessment of confounders would perhaps be illuminating, but given the number of conditions now found to have associations with MPV, a prohibitively large sample size would be required.

A key limitation of this study is that both those with embolic events prior to admission and those who developed emboli once admitted were counted in the outcome of embolic disease. Elevated MPV on admission may in part reflect activation occurring
in response to embolized occurring prior to admission. In order to better assess MPV as a prognostic marker for risk of embolus once in the hospital setting, a prospective study of large sample size would be valuable.

**Conclusions**

Mean platelet volume is significantly associated with embolic phenomena in infective endocarditis even when controlling for a broad range of demographic and clinical covariates. The association remains significant in patients suffering from embolic disease in IDU-IE.
CHAPTER 3: Handling of Missing Data

The impact that missing data has on outcomes depends on the type of missing
data. Data are classified as ‘Data Missing At Random (MAR)’, ‘Data missing Completely
at Random (MCAR)’, and ‘Data Missing Not at Random’. Data are considered
missing completely at random when the subset of people missing data are not statistically
different from the subset of those with complete data. This occurs when the probability of
having a missing data value is not related to any other patient characteristic. This could
happen when a survey is accidentally lost or a tube of blood is dropped on the floor. This
type of missing data is unlikely to alter data other than resulting in a decrease sample size
with all summary statistic measures being unaltered. Unfortunately, this type of missing
data is very rarely encountered in practice.

Frequently in research, data are NOT missing completely at random. Often one
subset of patients may be more likely to have missing data due to a specific characteristic
of that population subset. For example, if one is measuring blood pressure values over
time, the younger and healthier subset may be more likely to have missing follow-up data
due to the fact that they need a physician’s care less often. Though these data are not
MCAR, they may still be able to be considered MAR. Data are considered MAR when a
subset’s likelihood to have missing data can be entirely explained by other, measured
variables. Clearly, this terminology is quite misleading. It would seem to be against
the definition of the word ‘random’ to state that some other observed characteristic makes
one more or less likely to have missing data. The key is that the associated characteristic
is one the researcher has observed and measured. The researcher should perform a
thought exercise: if the patients are stratified by the variable(s) thought to explain
‘missingness’, would all groups have approximately an equal chance of being missing? For example: if one imagined only the subset of patient’s that were elderly and with high comorbidity, some would be missing data and others not. If one then imagined the subset of the young and healthy patient, some would be missing data and some not. When we look at the data within these stratified subsets, it is now random who is and is not missing data and these data can be considered MAR\textsuperscript{190,191}.

There is no statistical test to determine if data are Missing Completely At Random or are MAR. To formally test for a difference, one would have to know the distribution of values of the missing data to compare with the distribution of values of the known data. As by definition we do not know the values of the missing data- and have no way of ascertaining them- this comparison is impossible\textsuperscript{191}.

Data are classified as Missing Not At Random when the variables’ ‘missingness’ is related to UNmeasured variables. An example would be a survey among high school females that asks for a respondent’s body mass index (BMI) and eating patterns but those with a higher BMI turn out to be less likely to return their surveys. We have no way of knowing this since the BMI data, the data determining whether data are missing, is unobserved. There is a relationship between the propensity of the value to be missing and its own value\textsuperscript{190,193}.

Researchers have developed various statistical techniques to address missing data. Listwise Deletion (also known as complete case analysis) is where those cases without full data for all variables are simply dropped from the dataset\textsuperscript{192}. These cases are excluded from all analyses. This is a straightforward and simple solution but it results in multiple calculation errors if data are not MCAR. First, this may greatly deplete the
sample size and thus decrease power to detect differences; there is too much standard
error, lack of precision, in the results. Secondly it biases the various summary statistics of
the data such as the mean\textsuperscript{192,194}. The direction the summary estimates are biased, up or
down, depends on where the variables that were discarded lay in the distribution.

Pairwise deletion (also known as available-case analysis) is a technique where
incomplete cases are removed on a case by case analysis: you only remove those that are
missing the data you need for the specific analysis at the time. This results in fewer cases
being deleted which retains more power for calculations. However, this means that any
given case may contribute to some analyses and not others making interpretation
difficult. This too can result in biased estimates when the data is not MCAR.

Single imputation refers to sets of methods that seek to ‘fill in’ values for the
missing data. This has the benefits of resulting in a ‘complete’ data set for analysis and
retains all cases. There are multiple mechanisms of single imputation with some more
common than others. The first is the ‘mean imputation’. In this scenario, we fill-in
missing values for a variable with the mean of the available values for that variable. This
leads to multiple biases however. Having the exact same value for variable \(y\) (the mean)
with no regard to what the value of variable \(x\) is means that these values will show zero
association between \(X\) and \(Y\). The result is that the overall correlation estimate will be
too low. Also you have removed data variability which means that estimates such as the
standard deviation of the mean will also be too low. Finally, you will produce a biased
mean estimate for your sample; the direction of bias depends on if more missing values
were in the higher or lower tail of the sample. If more lower tail samples were missing
and filled in with the mean of values, these values are then higher than what would have
been truly present and the mean is inflated. The converse is true if more missing data were found in the upper tail.

Another common method of single imputation is regression imputation. In regression imputation, all of the variables with complete data are used as independent variables in a regression equation to calculate the variable with missing data; the variable with missing data is the outcome, Y variable. The result is an equation with parameter estimates that allow one to fill in an individual’s known values on the right-hand side of the equation and calculate the missing variable for that individual. The difficulty that results here is the opposite of that seen with mean imputation. Now the data fit perfectly on the line with a non-zero slope and no residual error. This OVER estimates the relationship between two variables and will make correlation estimates too high. Since we have replaced values that would have fallen at least slightly off of the line with values that are a perfect linear match to the regression line with no residual error, we have artificially decreased data variability and underestimated our standard error. This method produces unbiased measures of the mean itself but the standard deviation around the mean will remain artificially decreased and overstate the precision of the data. This can lead to increased Type 1 Error where a researcher identifies a relationship where none actually exists.

Stochastic regression imputation is a method which seeks to replace the variability that is lost in the single imputation methods explored above. Stochastic regression begins identically to regression imputation with the creation of a regression equation using complete variables as the Xs and the variable with missing data as the Y. The difference starts once the regression equation is formed because in this method a
randomly selected error term will be added to the end of the regression equation. This error term is randomly selected (by a computer program) from a normal distribution with mean 0 and a variance equal to the standard error of the regression line calculated from a complete-case regression analysis.\textsuperscript{190}

Multiple additional straightforward methods exist to fill in data. This includes ‘hot-deck’ imputation where missing values are filled in by values from a similar population. This could be a randomly selected individual from a pool of similar individuals or could be pulled from a group after patients are sorted into groups based on their similar covariates.\textsuperscript{195,196} One could also perform a sensitivity analysis whereby the data is filled in with a ‘worst-case’ and ‘best-case’ value. The results of the datasets are then analyzed to determine if they are statistically different.\textsuperscript{192} Finally, nonresponse weighting is an approach where one group with disproportionate amounts of missing data have their responses weighted to ensure that characteristics of the original population are retained. For example: if in a sample of 75% male and 25% female responses there are significant differences in the amount of missing data, female responses would have their responses weighted by $3 (0.75/0.25)$. This unfortunately works best when data are MCAR, an infrequent occurrence.

In recent years, advanced computer analytics have allowed researchers now to progress from more simplistic methods of single imputation to multiple imputation. This is considered the gold standard of missing data analysis. Multiple imputation analysis involves the steps of using an appropriate model to approximate the data, repeating the model over and over in a sequential manner using data filled in from previous iterations to create multiple datasets, and then pooling the results of these data sets to get estimates.
which are unbiased and with the appropriate amount of variability. A common approach starts with regression analysis. Complete data are used to calculate parameters for a regression model predicting the variable with missing values. Included in the equation should not only be covariates which will appear in final analysis markers, but also the auxiliary variables that support the idea that data are MAR. This model is then used to fill in the missing values. Unlike single imputation, with multiple imputation this process is then repeated: a new regression formula with new parameters is calculated from the data including the values which were imputed. This process is repeated iteratively until multiple data sets are obtained. Generally 5-20 sets are used depending on the variables and the amount of data. To introduce further independence into the datasets and to ensure that enough iterations have been run that an approximation of the ‘true’ missing data has been centered upon, 200 iterations or so are run between capture of each dataset. These datasets are then ‘pooled’ together to result in one final imputed value.

There are approaches other than imputation using formulas to account for missing data. K nearest neighbors (KNN) is a hot-deck imputation variation that is nonparametric- it does not require underlying assumptions about the data distribution unlike regression imputation models. KNN is at its heart a classification tool: it seeks to determine which data are similar to each other and can be grouped with each other. This classification system can then be leveraged to impute missing data.

KNN can be visualized as plotting each individual on a multi-dimensional grid with the number of dimensions determined by the number of covariates assessed. Those points that are found close to each other more closely resemble each other than those that
are far apart. Thus if one attribute of an individual is missing, we can look to those data points around it to make an educated guess about what the data should look like. KNN works by mathematically measuring and ranking the distance of each point relative to every other point to then determine which points can truly be considered ‘close’ and which ‘far’. There is actually more than one type of KNN distance function which can be incorporated to calculate distances between data points with different functions utilized with different types of data\textsuperscript{200}.

Once one has chosen a distance metric, one must still choose an appropriate K. Assessing only the single closest neighbor (K=1) risks selection of an outlier. By evaluating an increasing number of neighbors we get a better, aggregate view of the overall signal and decrease noise. Selecting too large of a K however reintroduces noise into the data. As K is steadily increased, one is moving steadily towards the farthest neighbor. By choosing a K, one is effectively drawing a line in the sand beyond which data points can no longer be considered ‘close’ and ‘similar’\textsuperscript{199,201}.  


Table IX: A Summary of Pros and Cons of Major Imputation Techniques

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Cons</th>
<th>Pros</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>• Decrease Power due to increased variance</td>
<td>• Simple</td>
</tr>
<tr>
<td></td>
<td>• Bias summary statistics</td>
<td></td>
</tr>
<tr>
<td>Mean Imputation</td>
<td>• Underestimates Variance</td>
<td>• Simple</td>
</tr>
<tr>
<td></td>
<td>• Biases Summary Statistics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Compromises Relationships between variables (usually underestimates)</td>
<td></td>
</tr>
<tr>
<td>Regression Imputation</td>
<td>• Underestimates Variance</td>
<td>• Unbiased estimate of the mean</td>
</tr>
<tr>
<td></td>
<td>• Biases association measures (usually overestimates)</td>
<td></td>
</tr>
<tr>
<td>Stochastic Imputation</td>
<td>• Still somewhat underestimates variance (less than those above)</td>
<td>• Unbiased parameter estimates</td>
</tr>
<tr>
<td>Multiple Imputation</td>
<td>• Complex</td>
<td>• Unbiased parameters</td>
</tr>
<tr>
<td></td>
<td>• Must ensure using base appropriate model</td>
<td>• Retains data variability</td>
</tr>
<tr>
<td>K Nearest Neighbor</td>
<td>• Lack of transparency</td>
<td>• Intuitive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Nonparametric</td>
</tr>
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</table>

In my data collection I had 19 unique individuals who had data for all variables EXCEPT for the MPV (table X). This provides an opportunity to assess the effects of different forms of imputation upon calculation of the MPV.
<table>
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<th>WBC</th>
<th>PLT</th>
<th>CR</th>
<th>ALB</th>
<th>Lg Veg</th>
<th>PE</th>
<th>PE or CNS</th>
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<tr>
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<td>Wake</td>
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</tr>
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<td>3</td>
<td>Wake</td>
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<td>0</td>
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</tbody>
</table>

Abbreviations: Age: in years; sex: -0 is male and 1 is female; Comorbidity: age-adjusted Charlson comorbidity score; Source: where the data continuous laboratory variable data came from; MPV: mean platelet volume; WBC: white blood cell count; Cr: creatinine; Alb: albumin; Lg Veg: any vegetation measuring >=10mm or described as a mass; PE: septic pulmonary embolus present if 1; CNS: central nervous system embolus present if 1; S aureus: Staphylococcus aureus was an involved pathogen if 1; L side: a vegetation was visualized on the left sided heart valves if 1
I performed mean imputation, regression imputation, stochastic regression imputation, and KNN with K set equal to 5 and 10. In order to prepare the data for the regression step, nonlinear variables were transformed to approximately normal. WBC was raised to the power of 0.25 with equal median and mean but Anderson Darling indicated lack of complete normality (A2= 0.002). Platelets were raised to the same power with similar roughly normal distribution but still not normal by Anderson Darling test (A2=0.01544). Creatinine was raised to -0.5 and albumin to 0.5 respectively with Anderson Darling measures of A2=0.028 and A2=0.09.

We can see that the range of variables filled in for each individual vary widely depending upon the imputation method employed (table XI). This then results in differing summary measurements and measures of variability for the variable MPV (table XII). Graphic representation of these measures shows that the summary statistics and measures of variability are unequal across imputation measures (Fig. 2)
Table XI: Individual Mean Platelet Volume Calculations Using Varying Imputation Techniques

<table>
<thead>
<tr>
<th>ID</th>
<th>Mean Imputation</th>
<th>Regression Imputation</th>
<th>Stochastic Regression</th>
<th>KNN K=5</th>
<th>KNN K-10</th>
</tr>
</thead>
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<tr>
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<td>9.1186</td>
<td>10.3378</td>
<td>7.9232</td>
<td>10.4333</td>
<td>11.175</td>
</tr>
<tr>
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<td>9.1186</td>
<td>10.4931</td>
<td>10.6396</td>
<td>10.95</td>
<td>10</td>
</tr>
<tr>
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<td>9.1186</td>
<td>9.3514</td>
<td>8.9993</td>
<td>10.6</td>
<td>10.3571</td>
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<td>18</td>
<td>9.1186</td>
<td>7.9075</td>
<td>8.032</td>
<td>7.925</td>
<td>8.2444</td>
</tr>
<tr>
<td>19</td>
<td>9.1186</td>
<td>8.7024</td>
<td>9.7247</td>
<td>7.75</td>
<td>7.875</td>
</tr>
</tbody>
</table>

Table XII: Summary Statistics of Mean Platelet Volume Calculated After Imputation Using A Variety of Techniques

<table>
<thead>
<tr>
<th>List Deletion</th>
<th>Mean Imputation</th>
<th>Regression Imputation</th>
<th>KNN K=5</th>
<th>KNN K-10</th>
<th>Stochastic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV Mean</td>
<td>7.3975</td>
<td>7.3975</td>
<td>7.3802</td>
<td>7.3684</td>
<td>7.3915</td>
</tr>
<tr>
<td>MPV MSE</td>
<td>0.0598</td>
<td>0.0484</td>
<td>0.0506</td>
<td>0.0501</td>
<td>0.0489</td>
</tr>
<tr>
<td>MPV Std. Dev.</td>
<td>0.5385</td>
<td>0.4841</td>
<td>0.5059</td>
<td>0.5011</td>
<td>0.4888</td>
</tr>
<tr>
<td>MPV Median</td>
<td>7.5</td>
<td>7.3975</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>

MSE: Mean Square Error  
Std. Dev.: Standard Deviation  
KNN: K nearest neighbor
When the results of these computations are inserted into the full dataset and the logistic regression model 3 is re-calculated, the parameters, odds ratios, and confidence intervals of the results are all changed (table XIII, Fig. 3). The MPV parameter estimate changed by at least 0.5 with the various regression techniques; stochastic regression resulted in the largest change (an increase of 0.1204). The changes in parameter estimates conferred an effect on the significance of the variable within the model. All imputation calculations resulted in a more statistically significant MPV value within the model. This is consistent with the data above showing that listwise deletion results in decreased power to detect associations. Stochastic regression resulted in the most significant parameter
estimate with a significance of <0.0001. As a consequence of these changes, the odds ratio point estimate for MPV also varied across imputation methods. All OR were higher for the imputed datasets than with listwise deletion with the confidence intervals shifted upwards. The largest shift was seen with stochastic regression where the OR became 1.6 with the upper limits of the CI approaching an OR of 2. Notably, the estimates and significance of the confounders also changed in the imputed models. This is seen largely with the variable of S. aureus. S. aureus failed to reach significance by a small margin in the listwise deletion model. It was, however, significant in all imputation models with a pointe estimate of about 0.5. This indicates that the odds of those without S. aureus as a causative agent having an embolic event are half those who do have S aureus as a causative agent.
Table XIII: Comparison of Logistic Regression Results When Missing Mean Platelet Volume Values are Imputed By a Variety of Methods

<table>
<thead>
<tr>
<th>Parameter Estimate</th>
<th>List Deletion</th>
<th>Mean Imputation</th>
<th>Regression Imputation</th>
<th>KNN K=5</th>
<th>KNN K-10</th>
<th>Stochastic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>0.002</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Odds Ratio</td>
<td>1.407</td>
<td>1.508</td>
<td>1.557</td>
<td>1.482</td>
<td>1.508</td>
<td>1.589</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.132-1.747</td>
<td>1.204-1.888</td>
<td>1.236-1.961</td>
<td>1.184-1.856</td>
<td>1.201-1.894</td>
<td>1.269-1.989</td>
</tr>
</tbody>
</table>

Covariates

<table>
<thead>
<tr>
<th>Source*</th>
<th>List Deletion</th>
<th>Mean Imputation</th>
<th>Regression Imputation</th>
<th>KNN K=5</th>
<th>KNN K-10</th>
<th>Stochastic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Veg**</td>
<td>0.432 (0.221-0.844)</td>
<td>0.401 (0.210-0.765)</td>
<td>0.404 (0.212-0.771)</td>
<td>0.411 (0.216-0.781)</td>
<td>0.407 (0.214-0.776)</td>
<td>0.393 (0.205-0.754)</td>
</tr>
<tr>
<td>Left Side#</td>
<td>2.380 (1.203-4.710)</td>
<td>2.436 (1.260-4.712)</td>
<td>2.459 (1.268-4.771)</td>
<td>2.399 (1.244-4.629)</td>
<td>2.414 (1.249-4.668)</td>
<td>2.557 (1.312-4.986)</td>
</tr>
<tr>
<td>S aureus##</td>
<td>Not Significant</td>
<td>0.515 (0.273-0.964)</td>
<td>0.508 (0.271-0.952)</td>
<td>0.502 (0.269-0.937)</td>
<td>0.499 (0.267-0.933)</td>
<td>0.513 (0.273-0.965)</td>
</tr>
</tbody>
</table>

*This is comparison of WFBMC as compared to those transferred from an OSH to WFBMC within 24 hours; No sig diff for those transferred within 24 hours and OSH

**Vegetations ≥ 10mm or described as mass on echo report; OR is a comparison of this being ABSENT

# The aortic and/or mitral valves were involved; OR is a comparison of this feature being ABSENT

## S aureus was the only or one of the causative microbes; OR is a comparison of this feature being ABSENT
Fig. 3 Trend of Mane Platelet Values by Analysis Type
In conclusion, there has been an evolution over time from more simplistic to more complex data imputation methods to account for missing data. These will become increasingly important for clinicians to understand as medical science becomes increasingly rigorous. In my data of MPV, the overall changes produced in the variability measures and mean are small. This resulted in small but still significant changes within my regression model across the varying techniques. In samples with increasingly large proportions of missing data these techniques will result in increasingly large outcome differences. Choosing the appropriate method to account for missing data is essential for complete and accurate analysis.
Citations


38. Luttgens WF. Endocarditis in main line opium addicts; report on 11 cases. *Arch Intern Med (Chic).* 1949;83(6):653-664.


175. Amjad AI, Parikh RA. Opana-ER used the wrong way: intravenous abuse leading to microangiopathic hemolysis and a TTP-like syndrome. *Blood.* 2013;122(20):3403.


APPENDIX

Appendix I: Measures and Strength of Association Between Nominal Variables

<table>
<thead>
<tr>
<th></th>
<th>Data Source</th>
<th>S aureus</th>
<th>Left Side</th>
<th>Large Vegetation</th>
<th>Drug Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S aureus</strong></td>
<td>χ²: 0.02</td>
<td>φ: 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left Side</strong></td>
<td>χ²: 0.01</td>
<td>φ: 0.20</td>
<td>χ²: &lt;0.0001</td>
<td>φ: -0.31</td>
<td></td>
</tr>
<tr>
<td><strong>Large Vegetation</strong></td>
<td>χ²: 0.01</td>
<td>φ: 0.20</td>
<td>χ²: 0.13</td>
<td>χ²: 0.0005</td>
<td>φ: 0.23</td>
</tr>
<tr>
<td><strong>Drug Use</strong></td>
<td>χ²: &lt;0.0001</td>
<td>φ: 0.44</td>
<td>χ²: 0.13</td>
<td>χ²: &lt;0.0001</td>
<td>χ²: 0.11</td>
</tr>
<tr>
<td><strong>Cardiac Surgery</strong></td>
<td>χ²: 0.29</td>
<td>φ: 0.20</td>
<td>χ²: 0.01</td>
<td>χ²: &lt;0.0001</td>
<td>χ²: 0.82</td>
</tr>
</tbody>
</table>

Appendix II Results of log-binomial regression Assessing Association Between Mean Platelet Volume And Embolic Phenomena of Endocarditis

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>SE</th>
<th>Significance</th>
<th>RR</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>-0.1609</td>
<td>0.0391</td>
<td>&lt;0.0001</td>
<td>1.1745</td>
<td>1.0879-1.2681</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.1020</td>
<td>0.0416</td>
<td>0.0141</td>
<td>1.1074</td>
<td>1.0208-1.2014</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.0544</td>
<td>0.0281</td>
<td>0.0527</td>
<td>1.0559</td>
<td>0.9994-1.1157</td>
</tr>
<tr>
<td>Model 4</td>
<td>-0.0738</td>
<td>0.0327</td>
<td>0.0238</td>
<td>1.0776</td>
<td>1.0089-1.1479</td>
</tr>
</tbody>
</table>

Abbreviations: LR: likelihood ratio; MPV: mean platelet volume β: parameter estimate for mean platelet volume SE: standard error for the mean platelet volume parameter estimate; OR: odds ratio; CI: confidence interval

Model 1 variables: age, gender, age-unadjusted comorbidity score, mean platelet volume
Model 2 variables: model 1 and white blood cell count, platelet count, creatinine, albumin, and data source
Model 3 variables: model 2 and atrial fibrillation and hypertension
Model 4 variables: model 3 and staphylococcus aureus involvement, left sided valve vegetation seen, large vegetation seen, injection drug use status
### Appendix III Results of Poisson Regression Assessing Association Between Mean Platelet Volume And Embolic Phenomena of Endocarditis (used poisson)

<table>
<thead>
<tr>
<th>Model</th>
<th>MPV $\beta$</th>
<th>MPV SE</th>
<th>MPV Significance</th>
<th>RR</th>
<th>OR 95% CI Lower</th>
<th>OR 95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1532</td>
<td>0.0518</td>
<td>0.0031</td>
<td>1.1655</td>
<td>1.0531</td>
<td>1.2901</td>
</tr>
<tr>
<td>2</td>
<td>0.1104</td>
<td>0.0630</td>
<td>0.0797</td>
<td>1.1167</td>
<td>0.9870</td>
<td>1.2634</td>
</tr>
<tr>
<td>3</td>
<td>0.1031</td>
<td>0.0639</td>
<td>0.1065</td>
<td>1.1086</td>
<td>0.9782</td>
<td>1.2565</td>
</tr>
<tr>
<td>4</td>
<td>0.1244</td>
<td>0.0656</td>
<td>0.0580</td>
<td>1.1325</td>
<td>0.9958</td>
<td>1.2878</td>
</tr>
</tbody>
</table>

Abbreviations: LR: likelihood ratio; MPV: mean platelet volume $\beta$: parameter estimate for mean platelet volume SE: standard error for the mean platelet volume parameter estimate; OR: odds ratio; CI: confidence interval

Model 1 variables: age, gender, age-unadjusted comorbidity score, mean platelet volume
Model 2 variables: model 1 and white blood cell count, platelet count, creatinine, albumin, and data source
Model 3 variables: model 2 and atrial fibrillation and hypertension
Model 4 variables: model 3 and staphylococcus aureus involvement, left sided valve vegetation seen, large vegetation seen, injection drug use status

### Appendix 4: Results of Poisson Regression with Robust Errors Assessing Association Between Mean Platelet Volume And Embolic Phenomena of Endocarditis

<table>
<thead>
<tr>
<th>Model</th>
<th>MPV $\beta$</th>
<th>MPV SE</th>
<th>MPV Significance</th>
<th>RR</th>
<th>OR 95% CI Lower</th>
<th>OR 95% CI Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1532</td>
<td>0.0338</td>
<td>&lt;0.0001</td>
<td>1.1655</td>
<td>1.0909</td>
<td>1.2194</td>
</tr>
<tr>
<td>2</td>
<td>0.1104</td>
<td>0.0406</td>
<td>0.0066</td>
<td>1.1167</td>
<td>1.0312</td>
<td>1.2093</td>
</tr>
<tr>
<td>3</td>
<td>0.1080</td>
<td>0.0413</td>
<td>0.0089</td>
<td>1.1141</td>
<td>1.0275</td>
<td>1.2080</td>
</tr>
<tr>
<td>4</td>
<td>0.1244</td>
<td>0.0428</td>
<td>0.0036</td>
<td>1.1325</td>
<td>1.0414</td>
<td>1.2314</td>
</tr>
</tbody>
</table>

Abbreviations: LR: likelihood ratio; MPV: mean platelet volume $\beta$: parameter estimate for mean platelet volume SE: standard error for the mean platelet volume parameter estimate; OR: odds ratio; CI: confidence interval

Model 1 variables: age, gender, age-unadjusted comorbidity score, mean platelet volume
Model 2 variables: model 1 and white blood cell count, platelet count, creatinine, albumin, and data source
Model 3 variables: model 2 and atrial fibrillation and hypertension
Model 4 variables: model 3 and staphylococcus aureus involvement, left sided valve vegetation seen, large vegetation seen, injection drug use status
CURRICULUM VITAE

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  o BS, BA, Louisiana State University, 2007
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    ▪ (Dr. Vera P. Luther, Program Director)
  o T32 Fellowship, Cardiovascular Medicine, Wake Forest University; 2016-present
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  o Clinical and Population Translational Sciences Masters Program, Wake Forest University 2016-present
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• CERTIFICATIONS:
  o American Board of Internal Medicine 2014
  o Infectious Diseases Board Certification 2016
• HONORS AND AWARDS:
  o Wake Forest KL2: Provides research funding to support 75% effort for a 2-year period to develop a tool capable of efficiently extracting injection drug use endocarditis cases from the electronic medical record.
  o Inaugural Wake Forest Internal Medicine Hazzard Scholar. In honor of Nascher/Manning Award for lifetime achievement winner Dr. William Hazzard, this award was created to nurture the early career development of clinician scientists committed to generating the new knowledge needed to improve patient outcomes and transform US healthcare delivery. The award provide participants with an individualized curriculum that builds the knowledge base and skills for productive and sustained careers as clinician scientists and support for 70% research effort over a two-year period.
  o Ike Muslow MD Visionary Award, Louisiana State University Health Sciences, 2011
• PROFESSIONAL DEVELOPMENT
- Fellow Immersion Training (FIT) in Addiction Medicine, Improving Research Skills for Subspeciality Fellow, R25DA013582, 2015

**BIBLIOGRAPHY:**
- ‘Rocky Mountain Spotless Fever?’ Presentation of a case of Rocky Mountain Spotless Fever without manifestations of rash. Powerpoint presentation delivered at American College of Physicians state meeting. 2013 Jan; Richmond, Virginia

**Active Research Support**
- DAL-301 DalCor Pharma UK Ltd.
A phase III, double-blind, randomized placebo-controlled study to evaluate the effects of dalcetrapib on cardiovascular (CV) risk in a genetically defined population with a recent Acute Coronary Syndrome (ACS): The dal-GenE trial

Reduction of SV mortality and morbidity in subjects with documented recent Acute Coronary Syndrome and the AA genotype at variant rs1967309 in the adenylate cyclase type 9 (ACD9) gene.

Role: Sub-Investigator

- D5881C00004 AstraZeneca
  A Long-Term Outcomes Study To Assess Statin Residual Risk Reduction With Epanova In High Cardiovascular Risk Patients With Hypertriglyceridemia (STRENGTH)
  A phase III, randomized, double-blind, controlled, parallel group design that will enroll patients with high triglyceride levels to study the efficacy of EpaNova (O3FA) in reducing the occurrence of major cardiovascular events.

  Role: Sub-Investigator

- AMR0101-0019 Amarin, Inc.
  A Multi-Center, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate the Effect of AMR101 on Cardiovascular Health and Mortality in Hypertriglyceridemic Patients with Cardiovascular Disease or at High Risk for cardiovascular Disease: REDUCE-IT
  Phase III, multi-center, prospective, randomized, double-blind, placebo-controlled, parallel-group clinical trial. The study’s primary objective is to evaluate the effect of 4g/day AMR101 for preventing the occurrence of a first major cardiovascular event.

  Role: Sub-Investigator

- Professional Societies
  - 2016-current American Heart Association
  - 2014-current Infectious Disease Society of America