Peer-Review

Arumilli, Sadhika, and Prasanthi Koneru. 2025. "Efficacy of the 'Popliteal Compression Test' in Detecting Intraoperative Popliteal Artery Transection Injury during Knee Arthroplasty in a Tier 2 Hospital in India: A Rapid Intra Op Clinical Test to Initiate Treatment Algorithm." *Journal of High School Science* 9 (3): 387–99. https://doi.org/10.64336/001c.143807.

1.This is a high school Journal. What was the contribution of the high school student to the project? Please note that a majority of the work (conceptual, experimental) needs to be preformed by the high school student. Was the manuscript written by the high school student?

2.The PPV is high and could lead to un-necessary vascular surgical procedures. You state "…..The one false positive case could have been due to a smaller genicular vessel branch which needed no treatment…." Does this mean that the vascular sugeon was not involved post replacement ? If not, please explain what then is the value of the test if a decision has to be made subjectively (with indication of bleeding) not to involve the vascular surgeon. Does this mean that < x mL of bleeding is considered not to involve the popliteal artery? Will this then not be dependent on the extent of damage to the genicular vessel branch? The squeeze test therefore no longer supports a binary decision of yes/no.

3.Assuming that the PPV extrapolates to larger populations, you will then subject 33 out of 100 patients to un-necessary vascular referral and surgery. Justify.

4.Your test involves forced pressure on the artery. Could there be instances where this forced pressure is enough to rupture a borderline-damaged artery; which could have healed by itself - had the popliteal squeeze test not been performed? Please discuss in detail in the manuscript along with recommendation to retrospectively compare patient outcomes before surgery is completed with and without the squeeze test. For example, if the incidences of iatrogenic injury to the artery were to be statistically greater in patients who were subjected to the squeeze test (with a large enough cohort size), than to those who were not so subjected; that would imply that the test itself was causing damage or rupture of the artery (or the peripherial arteries). Note that you have calculated based on expected true positives of 0.01%. However, literature reports suggest a few cases per 100,000 making the expected TP of 0.002% (lower end) (see references below).

5.Your statistical p values are based on your expected frequency values. Provide p values from a lower limit of 0.002% to an upper limit of 0.01% (see point 4 above).

6.Your procedure or methods do not include patient demographics. Please present in a table for those patients who tested positive for the test as well as those who tested negative. I realize this may be skewed but can yield valuable information to the reader of any underlying morbidities/age/sex/….. that were present in the failed cohort.

7.You mention a post operative review of 3 months but do not provide details or quantification. For example, did the review include a "….standardized examination procedure with ultrasound control, hemodynamic measurements, and a questionnaire regarding functional impairment……" You state "…..skin atrophy, loss of hair, nail atrophy and distal pulses….." This does not inform the reader of functional impairment.

8.You state that "……Any patient with previous vascular disease or vascular surgery and patients with known peripheral vascular disease were excluded……" This seems to be rather onerous criteria. Please

present the exact exclusion criteria. For example, were patients taking blood thinners excluded? Were patients with CVD excluded? ASA classification status? …… Are such exclusion criteira normally applied for this type of study? Provide references and justify.

9.Was ethics board approval obtained?
https://doi.org/10.1007/s00068-022-01961-8
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Authors response:

1. This is a high school Journal. What was the contribution of the high school student to the project? Please note that a majority of the work (conceptual, experimental) needs to be performed by the high school student. Was the manuscript written by the high school student?

A. Sadhika was actively involved in the initial brainstorming sessions that led to the formulation of the research question during her summer volunteer time at the hospital. Her curiosity about practical solutions to medical challenges in resource-limited settings, particularly in her home country, India, was a major driving force. She was keen in discussions regarding the clinical problem of vascular injury during knee arthroplasty and the need for a simple, effective diagnostic tool. Her insights helped shape the hypothesis that a straightforward clinical test could provide a timely solution, especially given the limitations of Tier 2 hospitals.

While direct surgical intervention is beyond the scope of a high school student's involvement, Sadhika played a crucial role in the observational and data collection aspects of the study. Under the direct supervision of Dr. Prasanthi Koneru, she was present during surgical procedures (observing from a safe, designated area) to understand the clinical context in which the Popliteal Compression Test was performed.

Her responsibilities included:

- Proforma Design and Data Entry
- Observation of Test Performance
- Follow-up Data Collection
- Analytical Contribution: Sadhika actively participated in the preliminary data analysis. She learned to organize the collected data, calculate basic statistical metrics such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). She was guided through the interpretation of these metrics and their clinical significance.
- Manuscript Writing: The initial draft of the manuscript, including the introduction, methods, results, and discussion sections, was primarily authored by Sadhika. The core narrative, the articulation of the problem, the description of the test, and the interpretation of the results were largely Sadhika's work. This hands-on experience in scientific writing was a key learning outcome of her participation.

This was finetuned by the senior author mainly the advanced statistics and all the crosschecking references. This project won the IRIS national fair 2024 award for research by a high school student and was selected for the ISEF 2025 final at Columbus, Ohio. This project provided sadhika with a comprehensive, real-world experience in medical research, fostering her scientific inquiry and technical writing skills, which aligns perfectly with the mission of the Journal of High School Science. We believe her

direct involvement in these critical steps of this project fulfils the journal's requirement for substantial high school student contribution.

2. The PPV is high and could lead to un-necessary vascular surgical procedures. You state "…..The one false positive case could have been due to a smaller genicular vessel branch which needed no treatment…." Does this mean that the vascular surgeon was not involved post replacement? If not, please explain what then is the value of the test if a decision must be made subjectively (with indication of bleeding) not to involve the vascular surgeon. Does this mean that < x mL of bleeding is considered not to involve the popliteal artery? Will this then not be dependent on the extent of damage to the genicular vessel branch? The squeeze test therefore no longer supports a binary decision of yes/no.

A. In the case of the single false positive, the Popliteal Compression Test was indeed positive, indicating fresh blood flow. As per our established protocol, any positive test result, regardless of the perceived volume or nature of bleeding, immediately triggers the involvement of the vascular surgeon. In this specific false positive case, the vascular surgeon was informed and consulted. However, upon subsequent immediate CT angiography, it was determined that the bleeding originated from a smaller genicular vessel branch and was not a significant popliteal artery injury requiring major surgical intervention. The decision not to proceed with a major vascular surgical procedure was made by the vascular surgeon based on the objective findings of the CT angiogram, not solely on a subjective assessment of the bleeding during the squeeze test.

The value of the test lies precisely in its ability to prompt this immediate, high-level consultation and objective diagnostic confirmation (CT angiogram), which is paramount in a setting where vascular expertise is not always immediately available on-site. The value of the test as a Screening Tool: The Popliteal Compression Test is designed as a screening tool, not a definitive diagnostic tool. Its primary value lies in its exceptional 100% sensitivity and 100% Negative Predictive Value (NPV). This means:

• No Missed Injuries, Early Alert System

Our observed PPV of 66.7% means that for every three positive tests, two genuinely represent a significant vascular injury requiring intervention, while one is a false positive. We acknowledge that this implies a proportion of patients (approximately 33% of those with a positive test, not 33% of all patients) will undergo an unnecessary vascular referral and subsequent CT angiogram. However, we strongly justify this approach for the following reasons:

- Catastrophic Nature of Missed Injury
- Minimal Risk of CT Angiogram
- Resource Optimization in Context
- No Subjective Decision to Exclude Vascular Surgeon
- No Volume Threshold (e.g., < x mL)

We would think of the popliteal compression test like fire alarms: I would rather evacuate unnecessarily a few times than miss a real fire once. The value of the test lies in its ability to trigger a rapid, life-saving diagnostic pathway, not in providing a definitive diagnosis on its own.

3. Your test involves forced pressure on the artery. Could there be instances where this forced pressure is enough to rupture a borderline-damaged artery; which could have healed by itself - had the popliteal squeeze test not been performed? Please discuss in detail in the manuscript along with recommendation to retrospectively compare patient outcomes before surgery is completed with and without the squeeze test. For example, if the incidences of iatrogenic injury to the artery were to be statistically greater in patients who were subjected to the squeeze test (with a large enough cohort size), than to those who were not so subjected; that would imply that the test itself was causing damage or rupture of the artery (or the peripheral arteries). Note

that you have calculated based on expected true positives of 0.01%. However, literature reports suggest a few cases per 100,000 making the expected TP of 0.002% (lower end) (see references below).

A. This is a crucial safety consideration for any clinical manoeuvre, and we have given it careful thought. We will incorporate a detailed discussion of this concern into the revised manuscript

   The Popliteal Compression Test involves a “milking manoeuvre” of the calf and thigh towards the popliteal fossa, followed by an anteriorly directed physiological force. The pressure applied is primarily aimed at displacing any residual blood within the venous and arterial systems distal to the tourniquet, forcing it proximally into the operative field if a transection is present. It is not a direct, forceful compression on the popliteal artery itself in a manner that would typically cause rupture of an intact or even a mildly compromised vessel.

   Several factors mitigate the risk of test-induced injury:

   1. Nature of the Injury: The injuries we are attempting to detect are typically transections or significant rents caused by surgical instruments (e.g., osteotomes, saws) during bone cuts or soft tissue release. These are acute, mechanical injuries, not subtle, borderline damages that would spontaneously heal or be susceptible to rupture from a gentle milking manoeuvre. A vessel that has been partially transected by a surgical instrument is already compromised; the test merely reveals the existing injury by demonstrating blood flow.
   2. Physiological Pressure: The pressure exerted during the squeeze test is comparable to physiological pressures experienced by the popliteal artery during normal muscle contraction or movement. It is significantly less than the pressure required to rupture a healthy artery. Even a borderline-damaged artery, if it were to rupture from such a manoeuvre, would likely be extremely fragile and prone to rupture from other minor intraoperative manipulations or even post-operative movement.
   3. Tourniquet Control: The test is performed while the limb is still under tourniquet control. This means that the arterial inflow is occluded, preventing high-pressure arterial flow from distending the vessel. The blood being mobilized is primarily residual blood distal to the tourniquet, not blood under systemic arterial pressure.

We agree that such a study, particularly with a large enough cohort, would provide definitive evidence regarding the safety of the Popliteal Compression Test. We will include this as a key recommendation for future research in the revised manuscript.

4. Your statistical p values are based on your expected frequency values. Provide p values from a lower limit of 0.002% to an upper limit of 0.01% (see point 4 above).

   The reviewer's question highlights a critical point that our observed incidence of true positives (2 out of 200, or 1%) is significantly higher than the reported literature incidence (0.002% to 0.01%). This discrepancy is likely due to our study focusing on a high-risk population (complex primary and revision knee arthroplasties) and the meticulous intraoperative detection method. Therefore, the chi-square test, when comparing our observed frequencies to these very low expected population incidences, would indeed show a statistically significant difference, meaning our observed rates are higher than the general population rates. Since the expected counts are very low, a Fisher’s Exact Test was also performed. Obtained a p-value of 1.00 (1.00>0.05).

   Instead of trying to force our observed data to fit a general population incidence, the more appropriate interpretation for our study is to focus on the diagnostic performance metrics of the test within our study cohort. These values are independent of the

population prevalence and accurately reflect the test's ability to identify vascular transection given that an injury occurs.

5. Your procedure or methods do not include patient demographics. Please present in a table for those patients who tested positive for the test as well as those who tested negative. I realize this may be skewed but can yield valuable information to the reader of any underlying morbidities/age/sex/…. that were present in the failed cohort.
   A: The demographic analyses like gender, age, BMI, type of surgery and co-morbidities are presented in the results section.

6. You mention a post operative review of 3 months but do not provide details or quantification. For example, did the review include a "…. standardized examination procedure with ultrasound control, hemodynamic measurements, and a questionnaire regarding functional impairment……" You state "….skin atrophy, loss of hair, nail atrophy and distal pulses…." This does not inform the reader of functional impairment.

A. We have used the Patient Reported Outcome Measures (PROMs) with Oxford Knee score template – Activities & Participation Questionnaire (OKS-APQ) at the end of 3 months for final follow-up.

7. You state that "……Any patient with previous vascular disease or vascular surgery and patients with known peripheral vascular disease were excluded……" This seems to be rather onerous criteria. Please present the exact exclusion criteria. For example, were patients taking blood thinners excluded? Were patients with CVD excluded? ASA classification status? …… Are such exclusion criteria normally applied for this type of study? Provide references and justify.

A. Patients were excluded from the study if they met any of the following criteria:

1. Previous Vascular Disease or Surgery: This included a history of diagnosed arterial or venous disease (e.g., atherosclerosis, deep vein thrombosis, varicose veins requiring surgical intervention, arterial aneurysms, or peripheral artery disease) or any prior surgical procedures on major blood vessels, particularly in the lower limbs.

2. Known Peripheral Vascular Disease (PVD): Patients with a clinical diagnosis of PVD, evidenced by symptoms such as claudication, rest pain, non-healing ulcers, or objective findings like diminished peripheral pulses, ankle-brachial index (ABI) < 0.9, or confirmed by vascular imaging.

3. Coagulopathies or Anticoagulant Therapy: Patients with known bleeding disorders (e.g., haemophilia, von Willebrand disease) or those on chronic anticoagulant therapy (e.g., warfarin, direct oral anticoagulants, clopidogrel) that could not be safely discontinued or bridged perioperatively. This was a critical exclusion to avoid confounding the interpretation of intraoperative bleeding.

4. Severe Cardiovascular Disease (CVD): Patients with unstable angina, recent myocardial infarction (within 6 months), uncontrolled arrhythmias, or severe congestive heart failure (NYHA Class III or IV). While not directly related to vascular injury during knee arthroplasty, severe CVD can significantly impact a patient's ability to tolerate prolonged surgery, anaesthesia, and potential vascular repair.

5. ASA Physical Status Classification > III: Patients classified as ASA (American Society of Anaesthesiologists) Physical Status IV or V were excluded. Excluding ASA IV and V patients ensures that the study population is relatively stable and can withstand the surgical procedure and any potential complications, thereby focusing on the test's efficacy rather than patient comorbidities.

6. Inability to Provide Informed Consent: Patients who were unable to understand or provide informed consent for participation in the study.

8. Was ethics board approval obtained?

A. Yes. it was obtained from the local institution.
IEC/IRB Ref No: 1085A-EC/1085A-01/24

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Thank you for addressing my comments. I have included some of your responses in the manuscript to add more depth and justification. Accepted.