Title: Investigation of the Quality Assurance Gaps in the Mouse Hindlimb Ischemia Model: Mapping Muscle Infarction and Regeneration with Angiogenesis Reveals Regional Variability

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Structured Abstract:

Background: Induction of hindlimb ischemia in the mouse is a widely used preclinical model for angiogenesis in the skeletal muscle. However, there has been little success in translating these results into clinical benefit for patients with peripheral vascular disease. Notably, the hindlimb skeletal musculature encompasses a vast territory, with heterogeneity in metabolic demands and blood supply. Accordingly, angiogenic responses following ischemia may be highly heterogeneous; however, this is unknown. This knowledge gap could compromise the clinical translation of this mouse model. The purpose of this study was to i) identify and map all muscle infarction/regeneration zones along with angiogenesis zones within the entire mouse ischemic hindlimb; and ii) determine the extent to which published studies have used an analysis strategy concordant with these findings.

Methods and Results: Thirty-two muscles from each hindlimb of four, 12-week-old C57BL/6 mice were studied, 10 days following unilateral femoral artery excision. Infarction/regeneration maps were generated for all muscles based on centralized myofiber nuclei. Angiogenesis zones were likewise mapped, based on a >20% increase in CD-31 immuno-positive capillaries relative to their contralateral uninjured muscle. Interestingly, only 15 of 32 muscles (47%) underwent infarction/regeneration. Furthermore, consistent uniform muscle infarction was seen in only five of the injured muscles (tibialis anterior bundle muscles), with patchy injury in the other 10 (including gastrocnemius and adductor muscles). Angiogenesis was evident in zones of infarction/regeneration but never in the non-infarcted muscles. Remarkably, there was little to no evidence for infarct "border-zone" angiogenesis. Subsequently, a systematic review identified 1,979 articles of which 160 publications were included for analysis. Here, only 15 of 160 (9%) reports evaluated angiogenesis in the tibialis anterior muscle bundle. Furthermore, assessment of angiogenesis in myofiber zones with centralized nuclei was evident in only 63 of 160 (39%) of studies, of which only 39 (24% of total) publications reported consistent central nuclei indicative of true consistent muscle infarction/regeneration.

Conclusions: There is extensive regional variability in skeletal muscle injury following femoral artery excision in C57BL/6 mice, with angiogenesis observed exclusively in infarction/regeneration zones. However, only a minority of published studies evaluated angiogenesis in the high-likelihood skeletal muscles or in regions of central myofiber nuclei. Developing standardized quality assurance parameters, based in part on these mapping data, could be critical to increasing translatability of mouse hindlimb ischemia studies.