G101 Postmortem Analysis of Vitamin D Using Liquid Chromatography Tandem Mass Spectroscopy

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The goal of this presentation is to review physiology of vitamin D, review current methodologies for measuring vitamin D, and understand the utility of measuring vitamin D in postmortem blood samples.

This presentation will impact the forensic science community by providing the framework to understand the utility of measuring vitamin D in postmortem blood samples. With the recent debate regarding vitamin D deficiency, bone fractures, and questions of child abuse it seems imperative to be able to address these issues as thoroughly as possible. Often is the case in forensic cases that antemortem blood samples are not available or specific questions have not been asked by a decedent's physician prior to death. Thus, there is no way to know if a vitamin D deficient state was present prior to death. The results study will allow the forensic community to know whether or not a postmortem blood sample can or cannot be analyzed appropriately for vitamin D nutritional status.

Objective: To measure vitamin D in postmortem blood samples using our recently developed liquid chromatography-tandem mass spectrometric (LCMSMS) method. Briefly, our current method provides for measurement of the 25-hydroxy derivatives of vitamin D, specifically 25(OH)-D_2/D_3, (OHD2, OHD3) in human serum. Increasingly, current clinical practice is to measure OHD_2 and OHD_3 to assess vitamin D nutritional status. To our knowledge, methods have not been evaluated for measuring these analytes in postmortem samples. The most common assay platform used today is an immunobased assay, which relies on antibodies which are known to cross-react with many vitamin D metabolites. Such immunobased assays are particularly sensitive to sample integrity and it is likely that a postmortem blood sample may not be appropriate due to hemolysis and other postmortem artifacts.

Hypothesis: Postmortem vitamin D concentration, measured with a sensitive and specific assay such as LC-MSMS, will correlate well with antemortem concentrations. Such analysis will be helpful in those cases where antemortem vitamin D levels have not been previously measured in the primary care setting. Furthermore, with the recent debate over vitamin D deficiency (Rickets) and suspicious non-accidental bone fractures, such an assay will, without doubt be of interest in cases questioning abuse.

Materials and Methods: In preliminary studies, three recent cases of natural disease were selected. In each case, peripheral blood (iliac vein) was sampled within 24 hours of the time of pronouncement. Approximately 8 ml of peripheral blood was drawn into a red-top tube

under gentle pressure to minimize hemolysis. Each sample was allowed to clot at room temperature for one hour and then centrifuged for twenty five minutes. The serum was then transferred to a clean red-top tube and frozen at -10 C until assayed. Hexa-deuterated OHD2 and OHD3 (OHD2d6 and OHD3d6, Medical Isotopes, Inc.) were used as internal standards (IS). Calibrators were prepared in acetonitrile (ACN) at 5, 10, 20, 50, 100 and 150 ng/ml for each analyte (OHD2 and OHD3). Samples and calibrators (500 ul) were spiked with 75 ng IS, extracted in 1 ml ACN and centrifuged. Thirty ul of supernatant was injected into a Shimadzu HPLC at 70% H2O:30% ACN at 350 ul/min flow. Analytes were separated on a C18 column (100 mm x 2.1 mm x 3 um, RESTEK) and then introduced into a triple quadrupole mass spectrometer (ABI 3200 Q-trap) via an APCI source in the positive ion mode. The analytes were eluted at 100% ACN over a 13 minute run.

Results: Preliminary studies addressed whether or not vitamin D analytes are stable in postmortem blood and if so whether they can be measured with our LC-MSMS method. In each of the samples tested to date, successful and reproducible total vitamin D in levels ranging from
6.43 ng/ml to 95.3 ng/ml have been detected and quantitated. We are confident in these results because the level of quantitation (LOQ) has previously established of these assay at 5 ng/ml.

Summary: It can be shown that postmortem blood contains measurable vitamin D and can be accurately measured on our LC-MSMS platform. Immediate planned studies on adult and pediatric cases include: (1) a direct comparison of hospital admission antemortem blood with our 24 hr postmortem blood samples; (2) a direct comparison of plasma and serum samples; and, (3) a postmortem stability assay to characterize how the postmortem interval affects our ability to accurately measure vitamin D.

Vitamin D, Postmortem Analysis, LC-MSMS

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