Molecular pathology selected abstracts

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Editors: Donna E. Hansel, MD, PhD, division head of pathology and laboratory medicine, MD Anderson Cancer Center, Houston; James Solomon, MD, PhD, assistant professor, Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York; Erica Reinig, MD, assistant professor and medical director of molecular diagnostics, University of Wisconsin-Madison; Marcela Riveros Angel, MD, molecular genetic pathology fellow, Department of Pathology, Oregon Health and Science University, Portland; Maedeh Mohebnasab, MD, assistant professor of pathology, University of Pittsburgh; Alicia Dillard, MD, associate clinical laboratory director, Omniseq/Labcorp, Buffalo, NY; and Richard Wong, MD, PhD, assistant professor of pathology, University of California San Diego.

Molecular autopsy to determine cause of sudden cardiac death

September 2025—When someone dies suddenly and unexpectedly, a forensic autopsy is often performed to identify the manner and cause of death. However, up to 30 percent of autopsies fail to identify any underlying anatomic or toxicologic etiologies, and the autopsy classification may be sudden unexplained death. This is particularly concerning in young people, in whom the cause could be an inherited genetic condition that could also affect family members. Therefore, postmortem genetic testing, also known as a molecular autopsy, may have utility in identifying underlying inherited diseases that could contribute to death. In many cases, sudden death is due to cardiac factors, but the type of cardiac dysfunction may depend on age. In adults over 35 years old, coronary artery disease accounts for up to 80 percent of cases of sudden cardiac death, while sudden death in younger people may be caused by dysrhythmias or structural abnormalities that often have a genetic component. The authors conducted a study, in Catalonia, Spain, in which they comprehensively analyzed sudden unexplained death in people younger than 50 years old. Between 2012 and 2023, they examined 2,039 cases in which the average age at death was 34.88 years. Most subjects were male (77.8 percent), and 70.1 percent of deaths were classified as cardiac related. The authors used different approaches for molecular testing based on age. For individuals under 35 years of age, they proceeded directly to genetic testing (n = 688). In this cohort, 7.6 percent of those studied harbored pathogenic or likely pathogenic variants, and those with cardiac causes of death had significantly higher positivity rates than those with noncardiac causes (9.5 versus 2.4 percent). For individuals between 35 and 50 years old (n = 1,161), genetic testing was performed only after the autopsy was complete and the death remained unexplained or suggested an inherited heart condition and after coronary artery disease was excluded. In the older study cohort, 4.9 percent of subjects were positive for pathogenic or likely pathogenic variants. Specific causes of death showing a particularly high genetic yield included ruptured thoracic aortic aneurysm and myocarditis, for which one-third of subjects in both cohorts harbored causative variants. In cases classified as sudden unexplained death, 8.9 percent of individuals were positive for pathogenic or likely pathogenic variants, with variants seen in genes associated with channelopathies or cardiomyopathies. Notably, no genetic variants were found in subjects who died from coronary artery disease, and only three percent of sudden infant death syndrome cases showed positive results. Overall, this study confirms that sudden death is more likely to have a genetic basis in younger people than in older people. The authors recommend molecular autopsy in all sudden death cases for people younger than 35 years old. It should be considered in those over 35 when the autopsy does not identify the cause of death, inherited heart disease is suspected, or death results from a ruptured aorta. Identifying

genetic causes of sudden death allows doctors to screen family members who might carry the same genetic variants, potentially preventing future tragedies through early detection and treatment.

Coll M, Alcalde M, Fernandez-Falgueras A, et al. Value of molecular autopsy in suspected sudden cardiac death in the young. *J Mol Diagn*. 2025. doi.org/10.1016/j.moldx.2025.05.006

Correspondence: Dr. Ramon Brugada at ramon@brugada.org

Comparing *ERBB2*/HER2 alterations in ctDNA and tissue of patients with metastatic urothelial cancer

The *ERBB2* gene encodes the receptor tyrosine kinase HER2, one of the most frequently altered targets in metastatic urothelial carcinoma. For years, HER2 has been successfully targeted in breast and upper gastrointestinal malignancies using monoclonal antibodies and small molecule tyrosine kinase inhibitors. Although these therapies have been less successful in metastatic urothelial carcinoma (mUC), a new class of treatments, called antibody-drug conjugates (ADCs), has shown promising pan-cancer activity in HER2-positive tumors, leading to new clinical trials in mUC. However, determining which patients may be eligible for these trials based on their HER2 status has been challenging in mUC, as testing guidelines and criteria are not as well established as for other tumor types. Moreover, previous studies have demonstrated variability in HER2 positivity among different testing methods and marked intra-patient heterogeneity in HER2 status. To understand the issues with determining HER2 status in mUC, the authors conducted an extensive retrospective analysis in which they examined 402 whole blood samples from 226 mUC patients and 244 formalin-fixed, paraffin-embedded tumor tissue samples. Cases were sequenced using a custom targeted panel covering more than 50 mUC-associated genes, including the entire coding region of *ERBB2*, to characterize somatic mutations, structural variants, copy number alterations, tumor mutational burden, and genome instability. *ERBB2* alterations were present in 15.7 percent of cell-free tumor DNA (ctDNA) samples, with protein-altering mutations in 14 percent of the entire study cohort and gene amplifications in 8.4 percent. Among the 28 patients with *ERBB2* alterations, *TP53* mutations were seen in 71.4 percent and *TERT* promoter mutations in 64.2 percent. *ERBB2* alterations and *FGFR3* alterations were mutually exclusive. A significant study finding was the remarkable spatiotemporal heterogeneity in HER2 status. For example, one patient developed a copy number gain of *ERBB2* and another lost a clonal *ERBB2* oncogenic variant after second-line pembrolizumab was initiated. Moreover, high sample-to-sample variability for individual patients was observed. In 15 percent of patients whose ctDNA was negative for oncogenic *ERBB2* alterations, tissue samples were positive, suggesting that relying on a single sample may be insufficient for assigning biomarker status. The authors also compared molecular testing results with IHC and found that 85 percent of tissue samples from patients with *ERBB2* alterations showed positive HER2 staining. However, almost half of patients without *ERBB2* alterations were also HER2 positive by IHC, suggesting nongenetic mechanisms of HER2 upregulation. Intra-patient heterogeneity in HER2 staining was also common, with 34 percent of patients showing IHC results varying between negative and positive depending on which sample was tested. Unlike in other cancers, in which HER2 alterations are typically early oncogenic drivers, *ERBB2* alterations in urothelial cancer may be later events only seen in a subset of tumor cells. Only 50 percent of tumors had *ERBB2* mutation with clonal prevalence in ctDNA, indicating HER2 variability within or between metastatic foci. This contrasted with other mUC oncogenes tested in ctDNA, such as *FGFR3*, which were predominantly clonal. This study highlights the complex biology of HER2 in mUC and suggests the use of multiple testing modalities for routine characterization of HER2 status. These findings are important because HER2-targeted therapies are of renewed interest for mUC treatment, with encouraging results in ADC trials. The unique mechanism of

ADCs may help overcome HER2 heterogeneity. Unlike prior agents that inhibit HER2 activity, ADCs only require the HER2 target to be present on a subset of cancer cells and deliver a chemotherapeutic payload that can result in bystander cell death and immune cell activation. Nonetheless, accurate assessment of biomarkers remains critical for implementing targeted treatment strategies.

Vandekerkhove G, Murtha AJ, Müller DC, et al. *ERBB2*/HER2 alterations in ctDNA and metachronous tissues of patients with metastatic urothelial cancer. *Clin Cancer Res.* 2025. doi.org/10.1158/1078-0432.CCR-24-3912

Correspondence: Dr. Alexander W. Wyatt at alexander.wyatt@ubc.ca, or Dr. Bernhard J. Eigl at bernie.eigl@bccancer.bc.ca, or Dr. Gillian Vandekerkhove at gvandekerkhove@prostatecentre.ca