Podcast Transcript

Episode 17: Decoding Time of Death: The Post-Mortem Interval and Toxicology Challenges

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TRANSCRIPT

Hi everyone, welcome back to The Tox Lab. I'm Rebecca.

I'm Rob. I don't know about you, Rebecca, but I love watching crime dramas.

Oh, I love them.

It's definitely a guilty pleasure of mine, but what I enjoy even more is pointing out all the bits that are wrong.

That is always fun when you know just enough to spot the nonsense.

Exactly. And one of my favourite examples is when they find a body, take one look at it, and immediately say exactly when the person died. So this week we thought we'd have a look at that phenomenon, how it affects us as toxicologists, and whether there are actually ways of determining when somebody has died with any real accuracy.

A bit of a health warning before we start: we are going to be talking quite a lot about death in this episode. Also, I'm not a forensic pathologist, so if there are any forensic pathologists listening who want to correct me on anything, please do get in touch. We'd love to hear from you.

So, post-mortem interval: what are we talking about? We're talking about the time that has elapsed since somebody died.

This can matter for a lot of reasons. It matters in legal cases, as in those crime dramas where someone finds a body and investigators need to know when the person may have died. But it also matters for toxicology. There have certainly been cases where toxicology findings may have been misinterpreted because the post-mortem interval was not properly considered.

So how do you estimate the post-mortem interval? One of the most obvious ways is from environmental clues. If somebody died at home, for example, things like when the milk in their fridge went out of date might help suggest when they were last alive.

But there are also some rough-and-ready physiological approaches, aren't there, Rebecca?

Yes. One is algor mortis, or body cooling. After death, the body gradually reaches the temperature of its surroundings. So if the body has not yet reached ambient temperature, you may be able to back-calculate, very approximately, when death occurred.

There's also rigor mortis, the stiffening of the body after death, which people are probably familiar with. This happens because ATP becomes depleted in muscle, and it follows a fairly predictable timeline. It tends to become fully established by around 12 hours after death, and then as decomposition begins, usually over the next 24 to 48 hours, it starts to resolve again.

So, based on the stage of rigor, you may be able to make a rough estimate of when death occurred.

Then there's livor mortis, or post-mortem hypostasis, where blood pools in the lowest parts of the body after the heart stops because of gravity. This causes a purplish-red discolouration. Again, it follows a timeline, starting roughly between half an hour and two hours after death and then becoming more established over time.

This is useful in the classic crime drama situation where they realise the body may have died in one position and been moved later, because once lividity becomes fixed, it doesn't just disappear.

Other approaches include forensic entomology, looking at the life cycles of insects on a body, which can help estimate how long a body has been there. There is also measurement of potassium in vitreous humour, the fluid in the eye, because potassium is known to rise after death and can potentially be used as a marker of post-mortem interval.

But all of these approaches are incredibly crude. There are so many variables involved in estimating the post-mortem interval that the error can be very large.

So Rebecca, why do we as toxicologists care about the post-mortem interval?

When we're looking at drug concentrations after death, there are many factors that can influence what we measure. Over the post-mortem interval, certain drugs redistribute depending on their properties and on where they are in the body. That can affect the concentration we measure, and it may not reflect what was actually present in the blood at the time of death.

Let's clarify what we mean by redistribution. We mean movement of drugs within the body after death, generally from areas of higher concentration to areas of lower concentration. A drug may have accumulated in an organ such as the liver during life, and then after death it can move into nearby blood, which is the very thing we later sample and analyse.

Exactly. After death, because there is no oxygen supply and no ATP production, the normal concentration gradients that existed during life cannot be maintained. Drugs can passively diffuse from tissues where they are concentrated into the blood.

At the same time, decomposition leads to loss of membrane integrity. You also get release of intracellular components and a shift toward anaerobic processes. Substances such as lactate can build up, the pH can fall, and the blood can become more acidic, all of which can further complicate interpretation.

And not all drugs behave in the same way, do they? This is very drug-specific, which makes it even harder.

Yes. Some areas of the body can effectively act as drug reservoirs. During the post-mortem interval, if a tissue contains a very high concentration of a drug, that drug may then diffuse into local blood vessels.

For example, tricyclic antidepressants can accumulate in the liver, so blood sampled close to the liver may contain higher concentrations than blood from a more peripheral site. Digoxin can be relatively concentrated in the heart, so cardiac blood may be higher than blood from peripheral sites.

Ethanol is another important example. If somebody drank alcohol before they died, the stomach may still contain ethanol, which can then diffuse into nearby vessels after death. That is one reason why we prefer more peripheral sampling sites, such as femoral blood, when interpreting post-mortem toxicology.

Some drugs are more prone to redistribution than others. Highly lipophilic drugs, meaning fat-soluble drugs, are often more affected than drugs that are more polar or water-soluble.

So a drug like clozapine is very prone to post-mortem redistribution, whereas a drug like morphine is generally less so.

Interestingly, drugs can also continue to undergo some metabolic change after death, which complicates things further.

One example sometimes discussed is amitriptyline being converted to nortriptyline in tissues where the relevant enzymes are present. And some drugs have very short half-lives anyway, so by the time we collect a sample they may no longer be detectable.

Heroin is a good example. The specific marker 6-monoacetylmorphine, or 6-MAM, has a very short half-life, so we often don't see it in post-mortem samples, which can make it harder to prove heroin use directly.

Bacteria can complicate things too. You can get post-mortem production of ethanol through fermentation of glucose, so it may look as though somebody drank alcohol before death when in fact some of that ethanol was produced after death.

That's something we need to consider when writing toxicology reports.

You can also get bacterial conversion of certain drugs, including nitrobenzodiazepines such as clonazepam, and those processes can vary with temperature and pH.

So in other words, there are a lot of factors that can affect drug concentrations during the post-mortem interval. Many of those factors will depend on how long elapsed between death and sample collection. If we had a better way of estimating the post-mortem interval, that could be really helpful for toxicology interpretation.

Yes. A lot of researchers have recognised that having a more accurate estimate of post-mortem interval would be useful for many reasons, including the ones we've just talked about. So we're going to look at a meta-analysis that examined mass spectrometry-based approaches to estimating the post-mortem interval using proteomics, metabolomics, and lipidomics.

So basically this means looking at proteins, metabolites, and lipids to see whether any of these markers change over time after death in a way that might help estimate the interval.

And omics approaches were very fashionable 10 or 15 years ago, weren't they? Large untargeted datasets where you don't necessarily know what you're looking for, but you're searching for patterns that distinguish one group from another.

Yes. This systematic review included 26 studies that met the inclusion criteria. Seventeen used proteomic approaches, nine used metabolomics, two used lipidomics, and one looked at all three together.

They covered different post-mortem intervals, broadly split into short, intermediate, and long intervals. Eleven of the studies looked at human tissues, while 18 used animal models, so that human-versus-animal gap is important when interpreting the results.

They also looked at a variety of tissues. For short-term post-mortem intervals, studies examined things like skeletal muscle, cardiac blood, lung, kidney, and liver. For intermediate intervals, some looked at spleen and soft tissues from different body regions. For very long intervals, the focus was mainly on skeletal muscle and bone, because beyond a certain point that may be all that remains.

Eleven of the papers produced predictive models for post-mortem interval based on the markers they identified.

So what did these studies actually find? Let's split it into short, intermediate, and long intervals.

A short post-mortem interval was generally defined as less than seven days. Proteomic studies in this category often focused on muscle tissue and identified proteins that seemed to decrease in a reasonably predictable way over the first few days after death. One example discussed was EEF1A2, which appeared to decrease over roughly the first 96 hours.

Some proteins may also become upregulated in relation to hypoxia and processes associated with cell stress, inflammation, oxidative injury, and apoptosis.

A lot of the metabolomics work in short post-mortem intervals was done in rats. In cardiac blood, some studies identified panels of metabolites that could potentially act as predictive markers within the first 24 hours. Xanthine and isoleucine were among the examples highlighted.

In skeletal muscle, some studies found dozens of compounds showing strong correlation with post-mortem interval over the course of up to a week.

So yes, there are a lot of possible compounds for this short time window, but none of them are in routine use at the moment.

Then there's the intermediate post-mortem interval, which in this paper meant roughly 7 to 120 days.

That's quite a wide range.

It is. In that category, a lot of the work focused on decomposition fluids in animal models. Researchers reported alterations in various peptides derived from proteins such as haemoglobin subunits, creatine kinase, and lactate dehydrogenase.

One problem is that a lot of these markers appear to be influenced by sex, body composition, and probably a range of other biological variables, which makes interpretation much harder.

So you'd need highly specific reference data across a very wide range of people to make this sort of approach genuinely useful.

And collecting that data would be a nightmare.

Completely. This intermediate interval was also the only stage where the review discussed lipidomics in any detail. Lipids such as stearic acid and palmitic acid appeared to increase during certain stages of decomposition, and unsaturated fatty acids may help mark transitions between those stages.

So there may be some value there as broad markers of decomposition stage, if not precise markers of time since death.

For the long post-mortem interval, meaning anything beyond about 120 days, the focus was mostly on bone tissue, because that may be all that remains for analysis. A variety of proteins, including histone-related markers and collagen-associated proteins, appeared potentially useful in distinguishing fresher remains from skeletonised material.

But from what I could gather, there still isn't an established marker in routine use that can accurately estimate post-mortem interval across real-world cases, because there are simply too many confounding factors.

You've got sex, age, body mass index, medical history, comorbidities, individual biology, and then environmental conditions such as temperature, humidity, where the body was found, and how decomposition progressed. All of those can affect these proteins, metabolites, and lipids.

So these omics studies have all identified possible markers. Did the review have a view on whether any of them really show promise?

The general conclusion was that this could potentially be a very useful area if the right marker, or more likely marker panel, could be found. But because of differences in study design, sample size, species, tissue type, and environmental context, it was very hard to draw strong conclusions about which approach was best.

So it isn't as simple as saying, if this marker goes up or down, then the person has been dead for exactly this long.

Not at all. Realistically you'd need a multi-factor model that accounts for all those other influences.

Which makes you wonder whether something like AI could eventually help, if you had enough data.

Maybe. As toxicologists, it would be great to have a simple analyte we could measure and then say, right, the post-mortem interval was approximately this, so we need to interpret the drug concentrations in that context.

That would be the dream.

It would. But at the moment the reality is much messier, and there is still a huge amount of uncertainty.

So at least for now, it still feels a bit like a fairy tale.

It does. AI may eventually help, but AI is only ever as good as the data you feed into it. And of course, you cannot ethically generate perfect human datasets for this kind of question.

Exactly. So even if AI ends up being useful, it would still be learning from imperfect and uncertain information.

So in summary, post-mortem interval estimation is a bit of a dark art, maybe even bordering on witchcraft, and certainly not like the television dramas where somebody looks at a body and announces that they've been dead for exactly 34.6 hours.

As toxicologists, it would be extremely helpful to have one or more robust markers that could tell us the post-mortem interval more accurately. But at the moment, that doesn't really seem to exist in a clinically or forensically dependable way. Maybe that will change in the future, and perhaps AI will contribute, but for now there is still a lot of uncertainty.

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Thank you so much for listening. Hope you all have a wonderful week, and we'll see you next time. Bye.