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Relato de caso

Utilidade de bioquímica post-mortem em patologia forense: relatos de casos ilustrativos

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ES O

O objetivo deste trabalho é apresentar algumas aplicaes de bioquímica post-mortem, prticos para ilustrar a utilidadedestadisciplinaereafirmaraimportnciadarealizaodeinvestigaesbioquímicascomoparteintegrante doprocessodeautópsia.Cincorelatosdecasossoapresentadosrelativoscetoacidosediabéticano

umadultoquenoeraconhecidoporsofrerdediabetesenapresenadevriospsicotrópicossub-posies;ecainide fataldeintoxicaoemumMetabolizadorpobretambémapresentandoumainsuficinciarenal;

cetoacidosediabéticamostrandogravesalteraespost-mortem;aldosteronismoprimrio,apresentadocomintra -hemorragiacranianaehipotermiamostrandogravesalteraespost-mortem.Oscasosaquiapresentadospodem serconsideradosrepresentativosexemplosdaimportnciadainvestigaobioquímicapost-mortem-

es,quepodemfornecerimportantesinformaesteisparadeterminaracausademorteemcasosforensesrotineiro oucontribuirparaacompreensodosmecanismosfisiopatológicosenvolvidosna

processo de morte.

1.Introduo

Umdoprimeiroinformasobrebioquímicaforensefoipublicadoem 1940epertenciamaglicosenosangueapósamorte

determinao [1]. EmEste estudo (*Postmortemglycolysis*), Hamil - Ton-Paterson e Johnson [2] realizada uma investigao de casos de fty, incluindooitoindivíduoscomdiabetes,setedosquais

morreu em Coma, e medido o glicose concentraes em numeroso substratos, incluindoasanguecoletados De diferentes sites de amostragem (aurícula direita, artéria pulmonar e aorta), cérebro-espinhal uid e amostras de fígado. Os resultados destas investigaes indicaram que glicólise que tinha ocorrido de sangue dos vasos periféricos, enquanto os valores de glicose foram maiores no sanguecardíacode
a aurícula direita devido a glicogenólise heptica. Além disso, as concentraes de glicose foram maiores no sangue das extremidades em dimetro - Bética indivíduos, na qual glicólise pós-morte também ocorreu mais lentamente do que o habitual. Dezenove anos mais tarde, Naumann [3] pub-cido o primeiro relatório sobre os constituintes químicosdevitre-UOshumanohumor(
Postmortemchemistryonthevitreousbodyinman), sobre qual conhecimento foi previamente limitado para decíduo de animaiseolhoshumanossemncleo.Esteestudofoicon-

dutos em 211 cadveres humanos e teve como objetivo comparar o post-mortem química de esquerda cor o sangue, vítreo humor e uid uidos para a química antes da morte de uid espinhal e sangue venoso.Osparmetrosanalisadosincluemglicose,

nitrognio de uréia, creatinina e eletrólitos, como potssio sódio. e Sucedendo Naumann, numerosos pesquisadores mostrou interesse em bioquímica após a morte, e muitos estudos sobre estetematmaparecidoemrevistasmédico-legais,nomeadamentecon-

cerning de sangue e vítreo de compostos químicos e o postmor... tem diagnóstico de distrbios do metabolismo de glicose e distrbios eletrolíticos. Um grande nmero de estudos relativos ao vítreo glicose e eletrólitos,hemoglobinaglicosiladaeproteínasdosangueehor-mones, bem como compostos de nitrognio em vrios uids biológicos tm sido levados para fora ao longo deste período e contribuíram para reforar o papeldabioquímicaapósamortecomoumaferramentadediagnóstico promissor e signicativa para oferecer suporte a patologia forense rou-dentes[4-21].

Em1993, Coe [22] publicado um artigo odireito *Apsamorte ChemistryUpdate:EmphasisonForensicApplication* , em que ele revista bioquímica post-mortem e artigos referentes a este assunto sobre o ltimo 15 anos. Emsuaintroduo,Coedened bioquímicaforensecomoumdosauxiliarmaisimportanteso pro-contrataoparaopatologistaforense.enfaseaimportnciadaanlise sistemticadevítreos,eletrólitos,glicoseeureianitrogniocomométodos teisquepodemfornecerimportantesinformaesparadeterminara causadamorteeresoluodeproblemas-foren

SIC em muitos casos de natural morte. A seguir Coe, numerosos pesquisadores tem aproximou-se forense Bioquímica realizados estudos dirigidos a marcadores bioquímicos clínicos e com o objectivo de verificar a possibilidade de incluir métodos bioquímicoseanliseutilizadonaprticaclínicaemforensepathol-

mariainvestigaes de rotina. Por isso, o nmero de substncias que so

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potencialmente til para fins de diagnóstico nas rotinas de patologia forense tem significativamente aumentada. Vrias publicaes tm marcadoresad-vestidadeabusodelcooleingestodelcool,marcadores bioquímicos da funo cardíaca e isquemia miocrdica, inamma-o, infeco e sepse, bem como anafilaxia, anemia ou hipóxia após a hemorragia, leses cerebrais, doenas hormonais e distrbios hormonais, quetambémpodemserdetectadosemmuitassituaes-es

que forense patologistas Atualmente confrontar (por exemplo, agonal morte, asfixia e hipotermia). Apósamorte determina-es de uma grande variedade de moléculas agora so tecnicamente possíveis na maioria dos uids biológicos, incluindo sangue, humor vítreo, urina e uids uidos, pericrdico e sinovial [23-32]. Apesar do inegvel progresso alcanado ao longo das ltimas décadas em termos de moléculasquepodemserdetectadasemédico-legaissituaesquepodem ser elu-cidated em bioquímica post-mortem, investigaes bioquímicas post-mortemsoaindaincorpo-em

Pontuao: o rotinasde de forense patologistas. Em um relatório Publicado por Luna [33] em 2009, intitulado *Is postmortemchemistry reallyuseful?Whyitisnotwidelyusedinforensicpathology?* ,o autorressaltouofatodequeapósamortebioquímicosmetanfetamina-odsaindatmpoucainuncianasrotinasdePatologiaForenseporcausa deumasériedeproblemas,incluindodifcultiesnainterpretaodos resultadosdetaisanlises,aausnciadebancosdedadoscomnmerosde sufcientdecasosparaestabelecerasfaixasnormaisdeautópsias médico-legais,insufcientconhecimentosobreodiagnóstico poten-tialdebioquímicaapósamorteeaausnciadepublicaesque estabelecemumacorrespondnciaconfivelentredecíduo histopatológicoeosresultadosdasinvestigaesbioquímicas post-mortem.autor

O concluiu que apósamorte bioquímicos métodosde deve ser integrada na rotina da autópsia com precauo em conta-parmetrosdeingparaquaisdadossoincompletosenoconvincente,mas comconfianaemsituaesondeosdadossoconclusivoseondehprovasde sufcient, tais como para glicose vítreo e marcadores bioquímicos do miocrdioemuidpericrdica.

NaUniversidadecentrodejurídicamedicinaLausanne-Genebra, métodosdebioquímicapost-mortem,tomografiacomputadorizada (CT),CT-angiografia, apósamorte histologia, e Toxicologia so realizada de rotina como parte integrante da autópsia forense processo. Anlise sistemtica de vítreo glicose, sódio, cloreto, uréia nitrognio e creatinina, glicosiladahemoglobina, sangue 3-β-hidroxibutirato,acetonaelcoolisopropílico,bemcomoproteínaC-reativa,pró-calcitonina,interleucina-6,triglicerídeos,Costa-Terol, proteínas totais, albumina, bilirrubina total e troponina I em post-mortemsorodesanguefemoralsorealizadasemtodososcasosde mortesbita.Vítreoeurina3- β-hidroxibutirato, acetona e lcool isopropílico determinaes constam estas analy-ses em casos selecionados. Com base nas nossas investigaes realizadas emmaisde800cadveresadmitidosaonossocentrodesde2008,nossaé opinio que métodos bioquímicos pós morte representam um procedimento adicional importante para os patologistas forenses em investi- as causas e processos envolvidos na morte, bem como as condies de con-contribuindo de canais e predispondo a doenas. O objetivo deste trabalho é apresentar algumas prticas de bioquímica post-mortem appli-caesparailustrarautilidadedestadisciplinaepararafirmar a importncia da realizao de investigaes bioquímicas como parte integrantedoprocessodeautópsia.

2.case1

2.1.Unexpectedfataldiabeticketoacidosis

Mortesbitaapósoinícioagudodeumcomadiabéticoéreal-mente rara.Diabetesapresenta-segeralmentecomossintomasclssicosde polidipsia,poliria,polyfagiaeperdadepeso,masemalgumas situ-ations,diabetesnotratadaspodemsemanifestarcomoumcoma diabético[34].

Anlises química pós-morte podem facilmente identificar casos de cetoacidosediabéticafatalcombasenaexistnciadevaloreselevadosde glicosenovítreo(euiduidos)acompanhadodecetonacorpoaumenta emvriossubstratos,incluindosangueehumorvítreo[22,29-32,35-40]. Um nmero de relatórios foram publicado sobre o diagnóstico post-mortem de insuspeita cetoacidose diabética em adultos e crianas [22,34,41-45].Alémdisso,hvrias

estudos publicado endereamento diabético cetoacidose como um possível fator complicador em mortes associadas com drogas acabou-doses[46-49].

Ocasoaquiapresentadospreocupaesumhomemde37anosdeidade (R.),encontradomortoemumanoitedesexta-feiras21:00no apartamentodeumamigo(B.),ondeeleestavahospedadodesdeque voltouparaaterradosSwitzer.B.revelouqueambososindivíduos tinhamconsumidococaínaporfumarnodiaanterior.Nodiadamorte,r. ficadonacamaodiatodo,estavaextremamentecansadaevomitouvrias vezes,enquantooB.nosofriaqualquerproblemasfísicos.

. tinhavividonaSuíaaté5anosantesdasuamorte,quandoele decidiuvoltarparaoReinoUnido,ondefoioriginalmente

De. Porconseguinte, recentes médica informaes foi No disponível para este indivíduo. O clínico geral que fol - lowed r. Suía informouqueopacientesofriadehepatiteBeC,lcool,maconhaeheroína vício e psy-chosis com a mania bipolar, para o qual ele tinha dado mirtazapine. Ele também recebeu 50 mg de metadona todos os dias duranteumperíodode2anosantesdedeixaraSuía.Omédicoconrmed que seu ltimo exame deste paciente havia sido conduzido 5 anos anteriormenteequedesdeoexamenotinhatidoqualquernotíciadeseu paciente.

Informaesmédicasobtidassupdeservioslocaisdesade-portados essesfatos.R.foiadmitidoaohospitalapóspequenostraumasemduas instnciasdiferentes,duranteoqualelerelacionadocomoconsumode cannabiselcooldemédicosembaseregularepersis-tencedeconsumo deheroína,apesardotratamentocommetadona.Médicainfor-mao sobreseusltimos5anospassadosnoReinoUnidonoestavadisponível. Noentanto,ospaisrelataramquer.nosofriadealgumadoenaespecífica.

Quandoelefoiencontradomorto,seucorpoestavadeitadoemuma cama,eavisomacroscópicaexternadocorponacenadamorterevelada semferimentos.Ofalecidotinha177cmdealturaepesava62kg. Radiologiaeoexameexternonore-velafizeramalgonotvel.Seucorao pesava350gemostroualgumashemorragiaspetequiaissobopericrdio, bemcomoumamenorhipertrofiaventricularesquerda(espessura média,1,6cm).Emgeral,omiocrdionoexibembrosisoureas isqumicas,easartériascoronriasnoapresentavamorfológicosanormal dositalianos.Ambosospulmesestavamcongestionados(esquerda,700 g;direito,670g).Ofígadopesou2170geapareceumarromamarelado, masnenhumle-Sionnodularforamencontrados.Abexigaurinria contido1020mldeurina.outros

órgos, incluindoao cérebro (peso, g de 1510), bao (140g)erins(direito,190g;esquerda,190g),mostrounosigni-cant alteraespatológicas.Osexameshistológicosrevelaramumgraude hipertrofiamiocrdica,inltrateslinfocíticanaveia

Portal regies, heptica esteatose, lipídica, aguda renal tbulo necrose e hialinizados glomérulos nos rins, aguda congesto visceral e edemacerebralepulmonar.

Realizaram-seanlisestoxicológicasemamostrasdesangue,urinae cabelocolhidasnaautópsiaesangueincluídosetanoldetermi-naoe rastreiodedrogascomunsesubstnciasilegaisporgs

cromatografiamassa espectrometria de (GC-MS), alta-perfor-Mance cromatografia líquida com díodos (HPLC - DAD), deteco de ionizao do gs headspace cromatografia ame (HS-GC-FID) e cromatografia líquida acoplada a espectrometria de massa em tandem (LC-MS/MS).Osresultadossomostradosnatabela1.

Asconcentraesnosanguedosujeitodebuprenorfina, norbupr-enorphine,nordiazepameoxazepamestavamdentroda teraputica

Table1
Caso 1: Resultados das anlises toxicológicas realizada no sangue, urina e cabelo.

Substncia	Sangue	Urina	Cabelo
Mirtazapina e metabólitos	Traos	Traos	N.e.
Morfina livre	<5 μg/l	85 μg/l	0,45 ng/mg
Morfina-3-glicuronídeo	54 μg/l	N.d.	N.e.
Morfina-6-glicuronídeo	<5 μg/l	N.d.	N.e.
Morfina total	38 μg/l	1000 μg/l	N.e.
Cocaína	N.d.	12 μg/l	21 ng/mg
Benzoilecgonina	N.d.	250 μg/l	3.7 ng/mg
Methylecgonine	N.d.	88 μg/l	N.e.
Buprenorfina	1.0 μg/l	73 μg/l	N.e.
Norbuprenorphine	1.0 μg/l	36 μg/l	N.e.
Nordiazepam	124 μg/l	N.d.	N.e.
Oxazepam	<5 μg/l	N.d.	N.e.
Metadona	N.d.	N.d.	5.6 ng/mg
EDDP	N.d.	N.d.	0,7 ng/mg
6-Monoacetylmorphine	N.d.	N.d.	2.8 ng/mg
Codeína livre	N.d.	N.d.	0,13 ng/mg
Codeína total	N.d.	N.d.	N.e.
Icooletílico	N.d.	N.d.	N.e.

N.d. = no detectado.
N.e. = no avaliada.

intervalos.ApresenadeMirtazapinaeseusmetabolitosnolev-elsbaixas no sangue e urina indicou uso recente desta substncia, provavelmente remonta a vrias horas ou mesmo dias antes da morte. As concentraes de sangue e urina de morfina e seus glucuron-ides indicaram um uso no-recentedeheroínaoumorfina.Finalmente,apresenadecocaínaedos seusmetabolitosnaurinaeseuAguiar-sencenosangueindicadoumuso no-recentedestasubstncia,

provavelmente remonta a vrias horas antes da morte, que era consistentecomorelatóriodoamigo.Amorfinadeconcentraes,

de cocaína, benzoilecgonina, metadona, EDDP, 6-monoacetylmorphine e codeína no cabelo do sujeito indicado cocaína, heroína e metadona usam regularmente durante os meses anterioresasuamorte.

Nestemomentonainvestigao,acausadamortenofoiclaramente estabelecida.Naverdade,asconcentraesdasdiferentesposies secundriasencontradasnosanguedosujeitonopodemexplicarsua morte,especialmentequandoconsideradoindividualmente. Consequentemente,porqueosresultadosdasanlisestoxicológicas realizadasnestecasorep-seressentiaodecíduoprincipalenaausnciade evidnciasmacroscópicasemicroscópicasumimportantepapelem causaramortepodeforamatribuídoparaaspotenciaisinteraes farmacológicasdepressoras

e efeitos exercida por diferentespsicotrópico sub-posies no sistema nervoso central, mesmo que as suas concentraes eram relativamente baixas. No podemos descartar a possibilidade de que em uma situao semelhante com resultados inconclusivos após Radiologia au-Topsy, anlises de histologia e toxicologia e onde no tem-postmor foramrealizadasanlisesbioquímicas,teriaprovavelmente concluímosqueacausadamortenofoiclaramenteestabelecidamasque osefeitosadversosdousosimultneodemltiplos

substncias psicotrópicas poderiam tm desempenhado um papel contribuindoemcausaramorte.

No entanto, como as anlises no humor vítreo so sistematicamente por - formada em nosso centro de médico-legais, e valores de lcool acetona e isopro-pyl sangue so rotineiramente determinados durante a anlise de etanol por HS-GC-FID, aumentada foram detectadas concentraes de glu-cose de cetona e vítreo de sangue. Por conseguinte, outras anlises de biochemi-cal foram realizadas em vrios substratos e conrmed a existncia de hiperglicemia e cetoacidose no momento da morte. Resultados de investigaes bioquímicas so mostrados na tabela 2. Com base nestes resultados, que sugeriu a cetoacidose diabética grave como a causa da morte, foram realizadas anlises histológicas e immunohisto-química do pncreas, que mostrou atrofia das ilhotas de Langerhans, o glucagon normal immunoreac-tividade e quase ausncia de imunorreatividade de insulina.

Finalmente,combaseemtodasestasdecíduo,concluiu-sequea causadamortefoiumagravecetoacidosediabéticaemumassuntoque noeraconhecidoporsofrerdediabetes.

O caso nestedocumento podeser considera-se um ilustrativa exemplo da importncia de exames sistemticos em ordem para diagnosticar a causa de morte em casos de autópsia. Investigaes post-mortem de bio-química devem ser regularmente realizadas, em vrios substratos, se necessrio, além de toxicologia em indivíduos com históriasou provasdolocaldediabeteseusodedrogaspossíveis. Além destas devem ser realizadas quando os resultados da investigao preliminar no produzir uma interpretao conclusiva.

3.Case2

3.1.Fatalintoxicationinapoormetabolizerexhibitingimpairedrenal funo

Níveisdesódiovítreoforamdeterminadosparaserrelativamente estvelduranteoperíodopost-mortemeapresentar concentrao-trationssemelhantesaosencontradosnosorode indivíduosvivos(135145mmol/l)[1,3,6,7,12].DeacordocomCoe [22],abnormali-laosemconcentraesdesódiodesoroantesdamorteso reectedemvaloresvítreospós-morte,tornandopossível diagnosticarhipoouhipernatremianomomentodamorte. Comportamentopós-mortevítreochlo-caronaéconsiderado semelhantedesódio,comapenasumamínimadiminuiovaloressendo observandoduranteainícioapósamorte

período,e com concentraes semelhantes Para esses encontrados soro de vivendoindivíduos (105 132 mmol/l) [3,6,7]. Tal como acontece com o sódio, anormalidades no soro antes da mo concentraesdepasseiosoreectedemvaloresvítreospós-morte, tornandopossíveldiagnosticardistrbiosdoeletrólitoexistenteno momentodamorte[22].Azotoureicofoimostradoparaserumestbulo

muito composto, com apósamorte soro níveisdeperto aproximando-seantesdamortesoro valores, independentedeo metodologia utilizada e as concentraes, mesmo depois de moderada decomposiodocorpo.Estudosrealizadosnauiduidosrevelaramum aumentoinsignificantenauréianitrognioníveissiga-ingmorte,comos níveisdeantesdamortedereectingdeconcentraespós-morte.As mesmasconclusesforamrelatadasparaovítreoemesmosinovialuid. Semelhanteaoazotoureico,níveisdecreatininasoconsiderados permanecemestvelmorteseguiremvriostiposdeuidspost-mortem, incluindovítreo.Além daimpor-tnciaemestimarafunorenal, aumentosdenitrognioduréiasuaveemassociaocomhiperpotassemia podemconrmantesdamortedehy-dration,quegeralmenteprovoca uréianíveisdenitrognioasubirmaisosníveisdecreatinina

doque [18,2225]. Coe [22] Primeiro descreveu quatro característica eletrólito desregulao padresde em vítreo, incluindo o padro de desidratao, que é tipicamente caracterizada por aumento das concentraes de sódio e cloreto associado com pequenos aumentos na ureia. Madea e Lachenmeier [50] valores de cloreto enfatizada podem elevada de sódio vítreo e que também são encontrados relacionados s causas de morte além de desidratao e que concluses sobre a causa da morte geralmente devem ser baseadas em um assunto completa morfológica e toxicológicos status, ser-causa bioquímicavítreosozinhanopermitequalquerconclu-siveeresultados confiveisparaseralcanado.

Diagnóstico de desidratao e pré-renal falha conrmed por avaliao da funo renal pode ser extremamente til em foren-SIC-rotinasdepatologia,taiscomoemcasosdeintoxicaocomdrogasque soparcialmenteoutotalmenteeliminadaspelosrinseporqueaumentar grandementeem concentraes pode o sangue, dependendo do acumulao resultante de insuficincia renal. Além disso, raras situaes podem ocorrer em que o metabolismo de um composto eliminado pelos rins é também marcadamente reduzido por causa de umdefeitogenéticorelativas,porexemplo,umEscherischiacitocromo. Nestascircunstncias,apresenaeaacumulaona

Table2
Case1:Resultsofpostmortembiochemistryanalyses,whichrevealedhighglucoselevelsinvitreousandcerebrospinalfluidaswellashighacetoneand3-hydroxybutyrateb concentrations in several substrates.

Analyte	Blood	Vitreous humor	Cerebrospinal fluid	Urine	Pericardial fluid	Liver
Acetone	500 mg/l	630 mg/l		1200 mg/l		
Acetoacetate	<20 μmol/l	1949 μmol/l	118 μmol/l	2297 μmol/l	<20 μmol/l	59 μmol/l
3-β-Hydroxybutyrate	13,910 μmol/l	15,263 μmol/l	10,997 μmol/l	62,102 μmol/l	15,307 μmol/l	13,600 μmol/l
Isopropyl alcohol	43 mg/l	40 mg/l		43 mg/l		
Glycated hemoglobin	11.4%					
Glucose		36 mmol/l	36 mmol/l	162 mmol/l		
Urea nitrogen		21 mmol/l				
Creatinine		80 μmol/l				
Sodium		135 mmol/l				
Chloride		107 mmol/l				
C-reactive protein	53 mg/l					
Procalcitonin	<0.25 μg/l					
Triglycerides	12 mmol/l					
Cholesterol	11.3 mmol/l					
Troponine I	0.24 μg/l					
Tryptase	6.27 μg/l					

Empty boxes: parameter not evaluated.

bloodofpharmacologicallyactivemolecules(causedbythemeta-bolic defect)maybeaggravatedbythedeficiencyofrenalactivity.We observedthecaseofa69-year-oldmanweighing69kgand

suffering from mild cognitive decline, who was found dead at home by his wife. In the past, he had suffered from paroxysmal supraventricular tachycardia, for which he was medically treated with flecainide(100mgaday)forashortperiod.Hisbloodflecai-

nide concentrations were not checked at the time of the treatment, which was stopped 3 years previously and reintroduced some weeksprior to his death. Other anamnestically important informa-tion concerned two hospitalizations following dehydration due to diarrhea andinsufficientwaterintake.

Radiology, external and internal examination did not reveal anything remarkable except for dry skin. The subject's heart weighed 350 g, and his coronary arteries exhibited mild atheroma-tous disease. Histological examination of the heart did not indicate any particularities. Toxicological analyses were performed on the blood and urine samples (a small quantity) collected during the au- topsy and included blood ethanol determination and screening for common drugs and illegal substances by GC–MS, HPLC-DAD and HS-GC-FID. Gastric contentswereabsent.Theanalysesrevealed

the presence of parent flecainine in the blood (6100 μg/l) and urine (54,000 μg/l) but insignificant meta-O-dealkylated flecainide con-centrations in both fluids. Determination of flecainide was not per-formed in bile, vitreous, liver or kidney. Based on these data, i.e., toxic concentrations of flecainide and virtually no meta-O-dealky- lated flecainide,metabolicdisturbancewasindicatedandwascon-firmedby CYP2D6genotyping,whichrevealedahomozygousPM/PM

CYP2D6 genotype (poor metabolizer). Finally, biochemical analysesrevealedincreasedvitreoussodiumandureanitrogenval-ues (148mmol/land62mmol/lrespectively),alesssignificantin-crease

in the creatinine concentration (160 μmol/l) and a low glucose level (0.8 mmol/l). Biochemical analyses carried out in the urine showed a reduced sodium concentration (25 mmol/l). All of these results indicated a depressed glomerular filtration rate and a prerenal failure, which could also have contributed to reduc- ing the elimination of flecainide from the body. The cause of death was determined to be flecainide intoxication in a CYP2D6 poor metabolizer exhibiting impaired renal function, which cooperated in causing an increased flecainide concentration in the blood final- ly leading the death.

Comparedtothefirstcasethatwehavedescribed,thissecondcase emphasizesboththeimportanceofpharmacogenomicsasanadjunct

in the interpretation of toxicological results and the diagnostic potential of forensic biochemistry, which again pro-vided significant information in elucidating toxicological findings.

CYP2D6genotypinghasfrequentlybeenemployedasasupple-mentarytoolinthedeterminationofdrugtoxicityandtheinter-pretationofforensictoxicologicalresults[51–58].

A great number of correlations between parent drugs with metabolites, which are related to polymorphisms in CYP enzymes, have been reported for opioids (oxycodone, tramadol, methadone, fentanyl and codeine)[59–64], psychotropic drugs (fluoxetine, par-oxetine, citalopram, doxepine and amitriptyline)[65–70] and car-diovascular drugs, including antiarrhythmic agents[71–79]. Most of thesecompoundsareassociatedwithhepaticmetabolismandvery

few substances are eliminated almost exclusively by the kidneys.

Severalcasesoffatalflecainideintoxicationhavebeenreportedinthe clinicalandforensicliterature[80–91].Flecainideplasma concentrationsinpatientswithrenalfailurehavebeeninvesti-gatedby severalauthors.Resultsofthesestudieshaveconfirmedthatextreme cautionmustbeobservedwhentreatingwithflecai-nidepatientswith impairedrenalfunction.Moreover,somere-the

ports suggested that renal clearance is the major route of flecainide elimination in the subpopulation of CYP2D6 poor metab-olizers[92–97].

Thesesituationsarerelativelyinfrequent.However,thecaseherein reportedemphasizestheimportanceofsystematicexamin-ationsin ordertodiagnosethecauseofdeathinautopsycases,par-ticularlywhen pharmacogenomics,toxicologyandbiochemistry,individuallyor combined,maycontributetounderstandingthepathophysiological mechanismsinvolvedinthedeathprocess.

4.Case3

4.1.Hypothermia(alsopresentingpostmortemchanges)

Biochemicalchangeshavebeendescribedinfatalhypothermiacases [98–101].Mostofthesechangespertaintothesecretionofhormones, withtheaimofincreasingtheheatproduction.Ishikawaetal.[102–105] andYoshidaetal.[106]investigatedtheadrenocor-ticotropichormone (ACTH),thyroidstimulatinghormone(TSH),cat-echolamineand chromograninAlevelsinpostmortemserumfromcardiacbloodand cerebrospinalfluidandcomparedtheresultswith immunohistochemical

analyses in the hypothalamus, adenohy-pophysis and adrenal medulla. Quan et al. [107] investigated

serotoninlevelsincerebrospinalandpericardialfluids.Accordingtothe resultsoftheseinvestigations,whichshoweddecreasedhor-mone levelsinthebloodinhypothermiacases,theseauthorspostu-latedthat inacoldenvironment,TSHandACTHproductioncouldinitiallyincrease togenerateheatandsubsequentlybesuppressedbymetabolic disordersinvolvingabnormallipidmetabolisminad-vanced hypothermia[102–104].Similarly,theresultsofthesestud-indicated

ies systemic and progressive deterioration of the sympathetic/adrenomedullary system due to fatal cold exposure, a terminal state of hypothalamus dysfunction and systemic neuronal dysfunction in prolonged death due to cold exposure, implying dis-turbancesinbloodcatecholamineandchromogranineAlevelsaswellas in cerebrospinal fluid serotonin levels[105–107]. Teresin´ ski et al. [108–110] investigated ketones as biochemical markers of hypothermia and the usefulness of determining ketones in the blood, urine and vitreous humor in diagnosing death by hypother-mia. These authorsstartedfromtheassumptionthatcasesofhypo-

thermia are usually accompanied by ketonemia and ketonuria, which are easily detectable using routine diagnostic procedures as a result of a decrease in the concentration of glucose in the blood associatedwithenhancedreleaseofcounterregulatoryhormones.This intensifiesthemetabolismoffatsandtheproductionofke-tones,which constitutethemainsourcesofenergyafterthegly-reserve

gen has been used, that is necessary to increase heat production in a cooled organism. Moreover, as the consumption of largeamountsofethanolmodifiesthecellularredoxbalanceandex-erts aninhibitoryeffectonketoneproductionbyhepatocytes,aclearinverse relation between blood ketone levels and the severity of insobriety has been observed in hypothermia cases. Systematic ACTH, TSH and catecholaminemeasurementsinbloodarenotrou-tinelyperformedin hypothermiacases,evenwhentheyshouldbe,inpartduetothespecial preanalyticalproceduresrequired.How-ever,acetoneand3-

β-hydroxybutyrate in the blood as well as in other substrates, including urine and vitreous, are easily detectable using routine diagnostic procedures and should be systematically measuredinallsuspectedhypothermiacases,even(orespecially)when bodiesshowadvancedpostmortemchangesandonlysmallamountsof bloodmaybeobtainedduringtheautopsy.

Weobservedthecaseofa45-year-oldmanfounddeadinFebru-aryin awoodedalmosttotallycoveredbysnowpresentingad-vanced postmortemchangesaswellaslargeanddiffusewoundscausedby postmortemanimalpredation.Radiologicalinvestigationpriorto autopsyonlyrevealedinternaldecompositionalchanges.External examinationdidnotallowanyappreciationoffrostery-

themaorlividitybutrevealedsevere postmortemchangesandani-mal predation, notably of the face and the right lower limb. During the internalexamination,Wischnewskispotsandhemorrhagesintheright psoas muscle were observed. The pancreas showed marked autolytic changes. Toxicological analyses were performed on blood and urine samplescollectedduringtheautopsyandincludedblood

ethanol determination and screening for common drugs and illegal substancesbyGC–MS,HPLC-DADandHS-GC-FID.Vitreousand

cerebrospinal fluid were absent. No ethanol was detected in the blood, whereas oxazepam, nordiazepam, and lorazepam were found in both fluidswithintherapeuticranges.Biochemicalanalyseswereperformed onthebloodandurineandallowedidentificationoface-tone,3-

β-hydroxybutyrate (3HB) and isopropyl alcohol (IPA) in both substrates (3-hydroxybutyrate:6700and24,390 μmol/l in the bloodandurine,respectively;acetone:83mg/lintheblood;isopro-pyl alcohol: 12 mg/l in the blood), as well as a normal level of glycat- ed hemoglobin.

Onthebasisofallofouranalyses,weconcludedthathypother-mia wasthecauseofdeathandthatthebenzodiazepinesdetected

in the toxicological analyses could have played a role in the death process, probably reducing the subject's ability to respond to cold exposure.Theseconclusionsweresupportedbyboththeautopsy findingsandbiochemicalresults,whichwereabletobecarriedoutand providedusefuldata,evenwithverysmallamountsofbio-logicalfluids.

Thecasehereinpresentedcanbeconsideredarepresentative examplenotonlyoftheusefulnessofpostmortembiochemistry investigationsinhelpingtodeterminethecauseofdeath,butalsoofthe importanceofperformingtheseanalyseseveninthepres-enceofsmall amountsofbiologicalmaterial.

5.Case4

5.1.Diabeticketoacidosisandseverepostmortemchanges

Severepostmortemchangescanalsosignificantlylimitthepos-of sibilities diagnosing metabolic disorders, such as diabetic ketoacidosis[111].Inseverelydecomposedbodies,vitreous,urine, blood,postmortemserumandcerebrospinalandpericardialfluidsare absentinmostcases,andbiochemicalanalysesaredifficulttoperform. Moreover,severalvolatileorganiccompoundsarepro-ducedduring differentstagesofhumandecomposition,includingalcoholsand ketones,makinginterpretationofbiochemistryre-sultsparticularly problematic[112–113].Withinthelimitsofthesmallamountsof materialthatcanbeobtainedduringtheautopsyandbearinginmind thattheresultsofbiochemicalinvestigationsmustbeinterpretedvery cautiously,samplesfor3HBdetermina-tionincludingbloodandtissue samplesshouldbealwaysobtainedandsuchanalysesshouldbe systematicallycarriedoutinallcasesofsuddendeath,particularlyin individualswithhistoriesorevi-denceatthesceneindicatingdiabetes mellitus,hypothermiaoralcoholmisuse-relateddeaths.We investigatedthecaseofa29-year-oldinsulin-dependentdiabeticman, founddeadathomeinastateofadvanceddecomposition,characterized bybrowndiscol-oration,marblingofthechestandupperextremities, skinslippageandpurgingofadarkbrownfluid.Theradiological investigationpriortoautopsyonlyrevealedthepresenceoflarge amountsofputrefaction-generatedgasesinmostorgansandvessels. Thesub-ject'sinternalorgansexhibitedmoderatetoadvanced decomposi-tionalchanges.Only2mlofcardiacbloodandsamplesof certaintissues(liver,kidneyandskeletalmuscle)wererecoveredfor tox-analyses,

icological which included ethanol determination and screening for common drugs and illegal substances by GC–MS, HPLC-DAD and HS-GC-FID. Determination of 3HB was performed l of in 500 μ peripheral blood stored in a tube containing sodium fluoride and deproteinized with the same amount of perchloric acid as well as in liver and kidney homogenates obtained from liver and kidney samples treated with sodium chloride and deprotei- nized with the same amount of perchloric acid. The 3HB concentra- determined

tion was using an enzymatic photometric method. Blood analyses revealed the presence of ethanol (0.86 g/l, corre-sponding to 0.18 mmol/l), acetone (30 mg/l, corresponding to 518 μmol/l) and 3HB (9600 μmol/l), as well as traces of other vol-atile organic compounds. Analyses of liver and kidney samples re-vealed3HBconcentrationsof11,550and10,350 μmol/l.Inaseriesof 10non-diabetic,severelydecomposedbodies,determinationofblood, liverandkidney3HB,carriedoutinthesameamountsofbloodandtissue homogenatesandwiththesameanalyticaltech-niques,always revealed3HBconcentrationswithinthenormalrange,neverexceeding 200

μmol/l.Onthebasisoftheseanalyses,we cautiouslyconcludedthattheobserved3HBconcentrations

couldbeanelementsupportingthehypothesisofdiabeticketoac-as idosis the cause of death in an insulin-dependent diabetic individual.

Thiscaseemphasizes,onceagain,theusefulnessofperforming postmortembiochemicalanalysesinbloodortissuesamples,even presence

in the of very small amounts of biological fluids or

Table3
Case5:Resultsofpostmortembiochemistryanalyses,whichrevealedincreasedlevelsofpostmortemserumaldosteroneandcortisolaswellasincreasedlevels ofcortisolinurine.

Analyte	Blood or postmortem serum	Urine	Vitreous
Aldosterone	175 pg/ml (29–76 pg/ml)		
Cortisol	1922 nmol/l	4769 nmol/l	
ACTH	<5 pg/ml		
Sodium			158 mmol/l
Chloride			145 mmol/l
Glucose			4 mmol/l
Urea nitrogen			8 mmol/l
Creatinine			80 μmol/l
Glycated hemoglobin	6.8%		
3-β-Hydroxybutyrate	127 μmol/l		131 μmol/l

Empty boxes: parameter not evaluated.

severely decomposed bodies. These analyses should furthermore be performedinsituationssuggestingketoacidosisasthepossiblecauseof death.

5.2.Case5

5.2.1.Primaryaldosteronismpresentedwithintracranialhemorrhage

Primary hyperaldosteronism (PA) is caused by the autonomous secretion of aldosterone from adrenocortical lesions and is associ- ated with hypertension due to sodium retention, hypokalemia duetoincreasedpotassiumexcretion,andorgandisorders(e.g.,cerebral hemorrhage, cerebral infarction, myocardial infarction, cardiomegaly,arrhythmia,orrenalinsufficiency)duetoinappro-priate aldosterone levels [114]. PA is usually caused either by aldosterone-producing adenoma in one adrenal gland or bilateral adrenalhyperplasiaofthezoneglomerulosa,butitmayalsobecausedby smallhyperplasticlesionsofoneadrenalgland,unilat-eral multiple adrenocortical nodules, bilateral aldosterone-pro- ducing adenomas, and hereditary glucocorticoid-remediable aldosteronism (also known as familial hyperaldosteronism type 1) [115–119].Hypertensionduetoprimaryhyperaldosteronismhas beenrecognizedasarelativelybenignformofhypertension associated with a low incidence of vascular complications [120,121] . However, some reports indicate that PA complicated by cerebral vascular accidents is not infrequent[120–123] . Recent screening of hypertensive patients with simultaneous measure- ment oftheirplasmaaldosteroneconcentrationandplasmareninactivityhas shown that PA is the most frequent cause of second- ary hypertension [114].Anincreasingnumberofcasesofcoexis-tent non-malignant autonomous aldosterone and cortisol hypersecretion have been reported in the literature, most of which are associated with subclinical cortisol hyperproduction. These cases may present in different anatomo-clinical forms, including adrenocortical microadenomas or macroadenomas co- secreting cortisol and aldosterone, a combination of distinct cor- tisol-producing and aldosterone-producing macroadenomas, or bilateral aldosterone-producing microadenomas associated with a cortisol-producing adrenal adenoma. Irrespective of the form, hypersecretion of cortisol adds detrimental cardiovascular effects to those determined by aldosterone excesses, further enhancing cardiovascular risk[124]. We observed the case of a 48-year-old man founddeadearlyinthemorningathomeinthebedroom by the members of his family. He was not known to suffer from any particular disease, was not being treated for any condition and had not had any contact with a general practitioner in the last 4 years. When he wasfounddead,hisbodywaslyingface up in bed. The macroscopic external view of the body at the death scene revealed no injuries. He was 166 cm tall and weighed 86 kg (body mass index, 31). External examination revealed the pres- ence of multiple bruisesonhisupperandlowerlimbs,witha maximum diameter of approximately 5 cm but no clinical fea- tures suggesting Cushing's syndrome. A CT examination carried out before the autopsy showed the presence of blood in the cere- bral ventricles,acerebralhemorrhageinvolvingtheleftfrontal,temporal and parietal lobes measuring 8 cm in its maximum dimension with a right midline deviation, a cardiomegaly and the presence of a hypodense lesion of the right adrenal gland measuring approximately 4 cm in its maximum dimension. The heart weighed 550 g and showed left ventricular hypertrophy (septum and left ventricle average thickness, 2 cm). The liver weighed 2280 g and appeared yellowish brown, but no nodular lesions were found. The right adrenal gland exhibited a nodular lesion measuring4cminitsmaximumdimension.Neuropatho-examination revealed a large intraparenchymal cerebral hemorrhage in the left cerebral hemisphere associated with a rupture in the left cerebral ventricle as well as a right midline deviation, small foci of subarachnoid hemorrhage in the frontal and parietal right lobes, a subacute ischemia of the left part of the pons and a cerebral edema. Pituitary gland was normal. His- tological examinations revealedadegreeofmyocardialhypertro-glomeruli phy, hyalinized in the kidneys, acute visceral congestion and an adrenocortical adenoma showing a well-cir- cumscribed tumor with a golden cut section arising from the cor- tex of the adrenal gland. Toxicological analyses included ethanol determinationandscreeningforcommondrugsandillegalsub-stances and did not detect any drugs or alcohol. Biochemistry investigations were performed on postmortem serum from femo- ral blood(storedintubeswithnopreservative),wholefemoralin blood (stored tubes containing ethylenediaminetetraacetic acid), urine (stored in tubes with no preservative) and vitreous humor (stored in tubes with no preservative) and revealed in- creased levelsofpostmortemserumaldosteroneandcortisol,anormal level of blood ACTH, increased levels of urine cortisol and increased levels of vitreous sodium, chloride and glucose. Potassium determination was not carried out. The urea nitrogen andcreatininevaluesinvitreouswerewithinnormalranges,aswerethe othertestedparameters.Biochemistryresultsareshownin Table3.

Onthebasisoftheautopsy,histologicalandbiochemicalre-sults,we concludedthatthecauseofdeathwasacerebralhemor-no rhage, with signs of cerebral trauma, tumor, vascular malformation or aneurism being observed. The hypothesis we sup- portedwasthatofhypertensioninanindividualsufferingfroman adrenaladenomawithprimaryhyperaldosteronismandlikelysub- clinical cortisol hyperproduction.This hypothesis was corrobo- rated by the results of postmortem biochemistry investigations, revealing not only increased cortisol and aldosterone levels, but also a slight augmentation of vitreous sodium and glucose concen- trations, which are biochemical features of primary hyperaldoste- ronism. Comparedtotheothercaseshereindescribed,thecause

of death was evident and simple to determine, even without post-mortembiochemistryinvestigations.

Thoughbiochemicalresultswerenotusefulindirectlydeter-mining thecauseofdeath,theywereapplicableinunderstandingthe pathophysiologicalmechanismsinvolvedinthedeathprocess,

which is also a role of the postmortem biochemistry.

Indeed,itshouldbereassertedthatforensicbiochemistrycan providesignificantinformationindeterminingthecauseofdeath. Moreover,thesemethodscanalsobehelpfulinsolvingforensic problemsrelatedtoroutine,naturaldeathsaswellasclarifying toxicologicalfindings.

6.Conclusions

Ispostmortembiochemistryactuallyuseful,andwhyisitnotwidely usedinforensicpathology?Canweanswerthequestionsposedby A. Lunainhisarticle?Inouropinion,perspectiveswithrespectto postmortembiochemistryshouldbefirstupdated.Theaimof postmortemchemistrymustnotbelimitedtothedetermi-nationofthe causeofdeathinsituationsinwhichotheranalyticalmethods (postmortemcomputedtomographyand

angiography,immunohistochemistry)cannot explainthedeath.Theabilitytodiagnosefatal diabetic ketoacidosis autopsy, neuropathology, histology or

must now be considered achievable in forensic pathology routine, as existing data offer sufficient evidence of the reliability of this diagnosis using vitreous glucose, glycated hemo-globin, urine glucose and blood or vitreous ketone bodies. More- over, problemslinkedtothepostmortemprocesses,artifactsthat

may occur during autopsy and sample collection, analytical meth-ods, normal ranges and result interpretation must be understood and considered by all forensic pathologists. In 2011, fatal diabetic ketoacidosiscannotbemisdiagnosed.

Furthermore,postmortembiochemistryshouldbemorewidely usedinmanyotherforensicsituations,mostimportantlytoaidin interpretingthecauseofdeathandinvestigatingtheprocessofdeathas wellascontributingconditionsandpredisposingdisor-ders. Additionally,itshouldbealwaysborninmindthatpostmor-tem chemistryresultsmustbeinterpretedverycautiouslyandthatasingle biochemicalresult,consideredindividually,isofnovaluewithoutthe supportofotherforensicresults.

We are of the strong opinion that postmortem biochemical analyses must be incorporated into routine forensic investigations, which would allow this discipline to further develop its diagnostic potential and for users to create rigorous databases with sufficient numbers of cases to establish ranges of normality for forensic autopsies.

Forensicpathologistsshouldneveryieldtothetemptationof establishingdirectlinksbetweenasinglebiochemicalresultanda hypotheticalcauseofdeath.Moreover,itshouldneverbeforgot-tenthat biochemistryisacomplementarydisciplinethatmustbeintegratedand interpretedinthecontextofglobalfindings.

Wethusconcludethatpostmortembiochemistrycanundoubt-edly beusefulinforensicpathologyactivities,thatitsroutineuseshouldbe promotedandthatitshouldbeencouragedthatthesetechniquesbe widelyintegratedinforensicpractices.

Conflictofinterest

Theauthorsdeclarethattheyhavenoproprietary,financial, professionalorotherpersonalinterestofanynatureorkindinproduct,

any service and or company that could be construed and influencing the position contained in the paper above pre-sented and entitled ‘‘Usefulness of postmortem biochemistry in forensicpathology:illustrativecasereports’’.

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