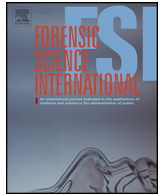




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The importance of microbiological testing for establishing cause of death in 42 forensic autopsies



S. Christoffersen*

Institute of Forensic Medicine, University of Southern Denmark, JB Winsløvsvej 17B, 5000 Odense C, Denmark

ARTICLE INFO

Article history:

Received 30 September 2014
 Received in revised form 3 February 2015
 Accepted 22 February 2015
 Available online 28 February 2015

Keywords:

Forensic autopsy
 Microbiology
 Cause of death
 CRP

ABSTRACT

Microorganisms have always been one of the great challenges of humankind, being responsible for both high morbidity and mortality throughout history. In a forensic setting microbiological information will always be difficult to interpret due to lack of antemortem information and changes in flora postmortem. With this study we aim to review the use of microbiological procedures at our forensic institute. In a retrospective study including 42 autopsies performed at our Institute, where microbiological test had been applied, analyses were made with regard to: type of microbiological tests performed, microorganisms found, histological findings, antemortem information, C-reactive protein measurement and cause of death. Fiftyone different microorganisms were found distributed among 37 cases, bacteria being the most abundant. Nineteen of the cases were classified as having a microbiological related cause of death. C-reactive protein levels were raised in 14 cases of the 19 cases, histological findings either supported or were a decisive factor for the classification of microbiologically related cause of death in 14 cases. As a multitude of abundant microorganisms are able to cause infection under the right circumstances, all findings should be compared to anamnestic antemortem information, before conclusions are drawn. A definite list of true pathogens is nearly impossible to compile.

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1. Introduction

Microorganisms have always been responsible for both high morbidity and mortality. Even today, new bacteria and viruses are discovered and old ones evolve to become a new problem, developing resistance to antibiotics or new lethal symptoms. Autopsies are important in the process of establishing cause of death [1], and though microbiological results and findings are often unspecific [2], they continue to be a valuable tool in the process of monitoring and identifying new infectious diseases [3]. Among the more recent are hanta virus pulmonary syndrome, West Nile virus, and severe acute respiratory syndrome (SARS) [4]. Especially bacteriological postmortem changes have been investigated through many years, dating back to Norris and Pappenheimer in 1905 [5] who introduced bacteria in the mouth of corpses and later showed they could be found in lung tissue at autopsy. The aim of this study was to review the use of microbiological procedures and findings at our forensic institute.

2. Methods and materials

In a retrospective study including all autopsy cases from our institute from the period from 1/1 – 2009 to 31/12 – 2011, a total of 669, 42 cases where microbiological testing had been applied were identified. These cases were examined with reference to: type of tests performed, detected microorganisms, histological findings, antemortem information, C-reactive protein (CRP) measurements and cause of death (COD). A Microsoft Access database was used.

If the antemortem information suggests febrile illness, localized infection, sepsis etc., a microbiological autopsy is usually performed. As soon as the sternum is removed 99% alcohol is applied, and with sterile instruments, the pericardium is opened and blood drawn from the heart with a syringe. Samples from both lungs are collected, using new sterile instruments for each lung. In cases of sudden unexpected death in infancy (SUDI) samples are retrieved from: heart blood, lungs, cerebrospinal fluid (CSF) and urine. If the examination indicates additional sampling, samples or swabs are collected from throat, middle ear, petechial hemorrhages of the skin, spleen, liver, thyroid gland, CNS, feces or other sites suggested by antemortem information or gross pathology findings.

Not all cases supply antemortem information suggesting a microbiological COD or infection, in such cases tissue samples

* Tel.: +45 65504460; fax: +45 65916227; mobile: +45 28794543.
 E-mail address: schristoffersen@health.sdu.dk

Table 1
Sampling sites and distribution of microbiological findings in cases with a microbiologically related cause of death.

	Sex	Age (years)	Cause of death	CRP (mg/L)	Sample site	Microbiological findings
1	Male	13	Viral pneumonia	N/A	Cerebrospinal fluid (CSF) Right lung Urine, Heart tissue, Thyroid gland, Left lung	<i>Candida albicans</i> , Coagulase-negative staphylococci <i>C. albicans</i> No growth
2	Male	40	Pneumonia	N/A	Pleura Right lung, Left lung, Blood	<i>Streptococcus pneumoniae</i> <i>S. pneumoniae</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i>
3	Female	2	Parainfluenza virus infection combined with Cytomegalovirus infection	<8	CSF Spleen Lung (side not specified) Blood, Feces	<i>Streptococcus salivarius</i> group, <i>Streptococcus mitis</i> group, <i>S. aureus</i> Cytomegalovirus Parainfluenza virus type 3, Non-hemolytic streptococci, <i>S. aureus</i> No growth
4	Male	55	Viral pneumonia	>160	Left lung Right lung Pericardial fluid	Influenza A(H1N1)pdm09, Influenza A non(H1N1)pdm, <i>E. coli</i> , <i>Acinetobacter</i> species, <i>C. albicans</i> Influenza A(H1N1)pdm09, <i>E. coli</i> , <i>Acinetobacter</i> species, <i>C. albicans</i> Influenza A(H1N1)pdm09
5	Male	46	Sepsis	>160	Blood, Right and left kidney Right lung, Left lung	<i>E. coli</i> <i>E. coli</i> , <i>C. albicans</i>
6	Male	39	Opiate poisoning combined with sepsis	>160	Right lung, Left lung, Heart blood	<i>E. coli</i> , <i>Clostridium septicum</i>
7	Male	33	Opiate poisoning combined with pneumonia	<8	Swap nose and throat	No growth
8	Male	84	Opiate poisoning combined with abdominal/kidney infection and pericarditis	46	Perirenal abscessus	<i>E. coli</i>
9	Male	65	Sepsis	>160	Right lung, Left lung, Blood, Mitral valve	<i>E. coli</i> , <i>S. aureus</i> , <i>Proteus vulgaris</i>
10	Male	55	Sepsis	>160	Feces Unspecified lymphnode Right lung Left lung Spleen, Heart blood Heart tissue	No growth β -Hemolytic group A streptococci β -Hemolytic group A streptococci, <i>S. aureus</i> , <i>C. albicans</i> , Non-hemolytic streptococci β -Hemolytic group A streptococci, <i>S. aureus</i> , <i>C. albicans</i> , <i>E. coli</i> β -Hemolytic group A streptococci Non-hemolytic streptococci
11	Male	39	Meningitis	N/A	Right lung, Left lung Heart blood Meninges	<i>C. albicans</i> <i>C. albicans</i> , <i>Enterococcus faecium</i> <i>Acinetobacter lwoffii</i> , <i>Pseudomonas fluorescens</i> , <i>Pseudomonas putida</i>
12	Female	67	Peritonitis	>160	Heart blood, Spleen	<i>Citrobacter</i> species
13	Female	54	Meningitis	147	Meninges Swap middle ear	<i>S. pneumoniae</i> Commeral flora
14	Female	34	Peritonitis	>160	Swap peritoneal cavity Spleen Right lung Left lung CSF, Feces	<i>E. coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacteroides fragilis</i> group <i>E. coli</i> , <i>B. fragilis</i> group <i>E. coli</i> , <i>B. fragilis</i> group, <i>Streptococcus parasanguinis</i> <i>E. coli</i> , <i>B. fragilis</i> group No growth
15	Female	81	Multiple fractures complicated by pericarditis	11	Right lung, Left lung Pericardial fluid	<i>Enterococcus faecalis</i> , <i>Hafnia alvei</i> , <i>Bacillus</i> species <i>E. faecalis</i> , <i>Streptococcus mitis</i> group
16	Male	80	Chronical heart decease and pneumonia	71	Right lung, Left lung	<i>E. coli</i>
17	Female	68	Sepsis	155	Right lung, Left lung Blood Spleen, Feces	β -Hemolytic group A streptococci, <i>E. coli</i> , <i>Clostridium</i> species β -Hemolytic group A streptococci, <i>E. coli</i> <i>E. coli</i> , <i>Clostridium</i> species
18	Female	67	Pneumonia	>160	Right lung	<i>S. pneumoniae</i>

and/or swaps are collected once suspicion is raised by gross pathology findings during the autopsy.

Tissue samples were analyzed at the Department of Clinical Microbiology, University Hospital of Odense. Samples are routinely analyzed for bacteria and fungi, using agar culturing. PCR analysis for viruses is performed per request.

CRP was measured in full blood using “QuickRead CRP” produced by Orion Diagnostica. The “QuickRead CRP” supplies results in the range of 8–160 mg/L, results below or above this interval will be noted as <8 and >160 respectively. Though an exact cut off value for CRP on postmortem material is not easily established [6], values above 10 mg/L are considered raised.

3. Results

The age ranged from 2½ months to 84 years, 8 cases were children under the age of 2, one was between 2 and 15 years old, 23 were between 16 and 60, and 10 were older than 60 years. Twenty-eight were male and 14 were female.

In five cases, no microorganisms were found. Bacteria were found in 36 (85.7%) of the cases, amounting to 247 of 354 (69.7%) samples taken. Viruses were found in 3 of 22 cases (1.4%). Fungi were found in eight cases (19.0%), amounting to 21 of 354 (5.9%) samples taken. No other named fungi than *Candida albicans* was found. In one case *C. albicans* was the only microbiological finding. Bacteria were found in 16 cases where they were not related to COD. Virus was only found in one case which was not classified as an infectious COD.

In the 42 autopsies COD was classified as infectious in 18 cases (42.8%), distributed among: three cases of viral pneumonia (16.7%), three cases of bacterial pneumonia (16.7%), four cases of sepsis (22.2%), two cases of meningitis (11.1%), two cases of peritonitis, one due to accidental perforation of the intestine during surgery and one due to perforated appendicitis (11.1%), one case of pericarditis (5.6%) and three cases of combined opiate poisoning and infection (16.7%), Table 1.

The COD of the remaining 24 cases, which were not related to microbiological findings, are listed in Table 2. In all, 52 different microorganisms were found including one report of “normal flora”, Table 3 shows the distribution.

Testing in one case, classified as viral pneumonia COD, did not yield any positive viral findings. However the histological findings were consistent with viral pneumonia (interstitial pneumonitis), and the person had been treated with antiviral antibiotics before death which could cause the negative viral findings [7].

CRP was measured in 33 cases. In nine cases CRP was below 8 mg/L. Regarding the 18 cases classified as infectious COD, CRP values were below 8 mg/L in two cases, 13 cases had elevated CRP values ($p < 0.05$), and in three cases no measurement was performed.

Table 2

Cause of death, in cases with no relation to microbiological findings.

Cause of death	Number of cases
Poisoning	5
SIDS	4
Cardiovascular disease	3
Raised potassium blood concentration	1
Ketoacidosis	1
Accidental strangulation	1
Embolia pulmonum	1
Exanginatio	1
Multi-organ failure	1
Bilateral adrenal gland bleeding	1
Eosiphil coronary periarteriteis	1
Unexplained	4

Table 3

Microbiological distribution in all samples.

Microorganism	Found in no. of autopsies	Found in no. of samples
<i>Acinetobacter baumannii</i>	1	6
<i>Acinetobacter lwoffii</i>	1	1
<i>Acinetobacter</i> species	4	5
<i>Bacillus</i> species	1	2
<i>Bacteroides fragilis</i> group	1	4
<i>Moraxella catarrhalis</i>	1	1
<i>Candida albicans</i>	9	20
<i>Citrobacter</i> species	2	4
<i>Clostridium butyricum</i>	1	6
<i>Clostridium septicum</i>	1	1
<i>Clostridium</i> species	1	4
<i>Corynebacterium</i> species	1	2
Cytomegalo virus	1	1
Enterovirus	1	1
<i>Enterobacter cloacae</i>	3	4
<i>Enterococcus faecalis</i>	3	4
<i>Enterococcus faecium</i>	2	2
<i>Escherichia coli</i>	19	61
Yeast	1	1
<i>Hafnia alvei</i>	2	6
<i>Haemophilus influenzae</i>	2	4
<i>Haemophilus influenzae</i> biotype 3	1	2
Influenza A(H1N1)pdm09	1	3
Influenza A non(H1N1)pdm	1	1
<i>Klebsiella oxytoca</i>	3	8
<i>Klebsiella pneumonia</i>	1	1
<i>Lactobacillus</i> species	1	1
<i>Lactococcus</i> species	1	1
<i>Neisseria</i> species non-meningococcal	2	2
Parainfluenza virus type 3	1	1
<i>Pediococcus</i> species	1	1
<i>Proteus vulgaris</i>	1	7
<i>Pseudomonas aeruginosa</i>	1	1
<i>Pseudomonas fluorescens</i>	1	1
<i>Pseudomonas putida</i>	1	1
<i>Pseudomonas</i> species	1	1
<i>Serratia liquefaciens</i>	1	1
<i>Serratia marcescens</i>	1	3
<i>Sphingomonas paucimobilis</i>	1	1
<i>Staphylococcus aureus</i>	6	21
<i>Staphylococcus intermedius</i>	1	1
Coagulase-negative staphylococci	9	13
<i>Stenotrophomonas maltophilia</i>	1	1
<i>Streptococcus mitis</i> group	3	3
<i>Streptococcus parasanguinis</i>	1	1
<i>Streptococcus pneumonia</i>	3	6
<i>Streptococcus salivarius</i> group	2	3
β-Hemolytic group A streptococci	2	12
β-Hemolytic group C streptococci	2	3
β-Hemolytic group G streptococci	1	1
Non-hemolytic streptococci	11	29
Commensal flora	2	2
No growth	5	79

Of the 18 cases with an infectious COD, histological examination was of importance in 14 cases (77.8%). In two of the 14 cases no microorganisms were found but the histological changes, CRP values and other information lead to the conclusion (one case of viral pneumonia and one case of neutrophilic pneumonia in an opiate poisoning case). In four cases, histological findings did not show inflammation to support the microbiological findings, however CRP levels above 160 mg/L and the bacteriological results were sufficient to verify the microbiologically related COD.

4. Discussion

Culturing bacteria on agar plates is an old invention whereas polymerase chain reaction (PCR) used for viral detection is a fairly new technique and is still costly compared to agar cultures. Hence bacteria and their colonization of the body is the most thoroughly

investigated group of pathogens. A single study has used PCR for bacterial detection with excellent results [8]. Though this might be the way to proceed in the future, the skill and equipment required for PCR, compared to using agar plates, could mean that it will not be the investigation of choice in the near future on a worldwide basis.

The key point in interpreting postmortem microbiological results is to determine whether detected microorganisms arise from postmortem spread or are the result of true antemortem infection. Larsen et al. suggest that to be sure a bacteria was present in the bloodstream before death, it must be cultured from at least two different sites [9]. However authors do not agree on the most reliable sites for sampling, blood, lung and spleen are generally mentioned, as well as cerebrospinal fluid [10,11]. Tuomisto et al. [8] suggest liver and pericardial sampling as the most reliable within the first five days after death. Preferably, cultures should be verified by gross pathologic or histologic findings [7,9].

When bacteria are cultured postmortem, one must consider several mechanisms which could lead to bacteria in the samples. Four lines of spread have been suggested [7,12].

1. Invasion in life: A microorganism has genuinely invaded the bloodstream or target organ during life leading to bacteraemia/infection/sepsis, the group of cases which could be classified with a microbiological COD.
2. Agonal spread: According to this theory the ischemia in the agonal period or during resuscitation lead to mucosal damage allowing bacteria to invade through the surface in question.
3. Postmortem translocation: The movement of naturally present bacteria across the mucosal barriers, into the surrounding tissue, bloodstream and body. One should expect a mixed growth and not a single isolate bacteria [13].
4. Contamination: Microorganisms are introduced into the tissue at time of sampling, and were not present in life.

Table 4
Microbiological findings in cases with no clear COD.

Sex	Age (months)	Cause of death	CRP (mg/L)	Sample site	Microbiological findings	Histology
Male	8	SIDS	N/A	Right lung	<i>Haemophilus influenzae</i> biotype 3, Non-hemolytic streptococci, β -hemolytic group G streptococci, <i>Moraxella catharralis</i> , <i>Pediococcus</i> species	No inflammation
				Left lung	<i>Neisseria</i> species non-meningococcal, Non-hemolytic streptococci, β -hemolytic group C streptococci, <i>H. influenzae</i> biotype 3	No inflammation
				Heart tissue Heart blood Brain tissue, Cerebrospinal fluid (CSF), spleen	<i>Citrobacter</i> species <i>Citrobacter</i> species, <i>Klebsiella pneumoniae</i>	No inflammation No relevant histology
				Feces	No growth	No inflammation
Male	8	SIDS	<8	Right lung	<i>H. influenzae</i> , Non-hemolytic streptococci	No inflammation
				Left lung	<i>H. influenzae</i> , Non-hemolytic streptococci, Coagulase-negative staphylococci	No inflammation
				CSF	No growth	No relevant histology
				Heart blood	Non-hemolytic streptococci	No relevant histology
				Spleen	No growth	No inflammation
				Non specified lymphnode Heart tissue	Non-hemolytic streptococci <i>Enterobacter cloacae</i>	No inflammation No inflammation
Female	2½	SIDS	N/A	Right lung	<i>Klebsiella oxytoca</i> , <i>E. cloacae</i> , Non-hemolytic streptococci	No inflammation
				Left lung	Coagulase-negative staphylococci, <i>K. oxytoca</i> , Non-hemolytic streptococci	No inflammation
				Lymphnode throat Spleen	<i>K. oxytoca</i> , <i>Acinetobacter</i> species <i>K. oxytoca</i>	No relevant histology No inflammation
				Right lung, left lung, spleen Heart blood, peripheral blood, Heart tissue	No growth No growth	No inflammation No inflammation
Male	19	Unexplained	<8	CSF, brain tissue	No growth	No inflammation
				Swap from nose and throat	No growth	No relevant histology
				Undefined tissue 1 Undefined tissue 2	<i>K. oxytoca</i> <i>Escherichia coli</i>	No relevant histology No relevant histology
				Lymphnode abdomen Spleen Heart tissue	<i>E. coli</i> , <i>Enterococcus faecalis</i> , <i>Lactobacillus</i> species <i>E. coli</i> <i>E. coli</i> , <i>Acinetobacter</i> species, Coagulase-negative staphylococci	No relevant histology No inflammation No inflammation
Female	13	Unexplained	<8	Left lung	<i>E. coli</i> , Coagulase-negative staphylococci, <i>Streptococcus mitis</i> group	No inflammation
				Right lung Blood, CSF, Feces, swap from nose and throat	<i>E. coli</i> , Coagulase-negative staphylococci No growth	No inflammation No relevant histology
				Blood Left lung	Enterovirus Non-hemolytic streptococci, <i>Corynebacterium</i> species	No relevant histology No inflammation
Female	11	SIDS	<8	Right lung Heart blood	No growth Non-hemolytic streptococci, <i>Corynebacterium</i> species, <i>E. coli</i>	No inflammation No relevant histology
				Liver, spleen CSF, swap from middle ear	No growth No growth	No inflammation No relevant histology

The theory of agonal spread is according to a review by Morris et al., considered unlikely [13]. Several papers find that elapsed time from death to sampling point does not have a significant influence on false positive bacterial results provided that bodies are kept refrigerated [11,13–15]. However animal experiments performed by Heimesat et al. [16] do show a rather large increase in bacteria in extra-intestinal tissue especially up to 72 h after death. Perhaps even more interesting, they observe a rise in extra-intestinal bacteria already five minutes after death. Maujean et al. report an interesting case in which *Neisseria meningitidis* was detected in a putrefied body after 10 days using molecular analyses [17]. Pryce et al. compared antemortem vs. postmortem sampling and concluded that postmortem sampling is as valuable as antemortem, when related to an infectious COD [18]. The risk of contamination from the autopsy room seems to be small [19].

Table 3 shows the vast diversity of microorganisms found in our cases, and illustrates the large number of possible pathogens. The majority of bacteria could be considered postmortem translocation, however as many of the microorganisms contributing to our normal flora can become pathogens under the right circumstances, all findings must be carefully considered.

In our study bacteria were found in 36 cases, 16 of these cases were not classified as having infectious COD, adding to the presumption of a significant postmortem spread. The vast majority of the microorganisms found in these cases are part of our natural flora, and are to be considered opportunistic pathogens, several being able to cause both pneumonia and bacteremia. In six of these 15 cases no clear COD was found, four were SIDS and two unexplained under the age of two (Table 4). Histology did not show inflammation in any of the samples, and none of the cases had elevated CRP values, though in two cases CRP measurement was not performed.

Not surprisingly, these problematic cases are all infants. As several of the bacteria are possible pathogens, infection cannot be completely ruled out due to the fact that a not fully developed immune system could be responsible for the lack of both inflammatory response and rise in CRP values.

Several more or less commonly encountered microorganisms, such as *N. meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Salmonella* species, *Staphylococcus aureus*, *Streptococcus pneumoniae*, β -hemolytic streptococci, *Klebsiella* species, *Escherichia coli*, *Mycobacterium tuberculosis*, members of the Enterobacteriaceae and *C. albicans* should, according to some authors, always be considered pathogens [10,11,13]. Several of the above are however part of our natural flora, and may be considered opportunistic pathogens [20], only causing disease in persons with an underlying illness, immune deficiency, or are perhaps only known to cause disease in children etc. [21]. Generally all cases should be correlated to anamnestic information, if any present, both medical and socio-economic [22] before a microorganism is classified as a true infection. Hence a definite list of true pathogens is impossible to comprise.

Considering that microbiological testing amounts to only 6.3% of the autopsies in the investigative period versus the yield of cases which were classified as microbiologically related COD (42.8%), this inexpensive testing seems to be rather well founded.

Perhaps microbiological testing should be applied in even more cases. In Denmark around 5% of autopsies do not yield a viable cause of death, and though microbiological testing would be performed on most, the possibility remains that a few of these deaths could be due to infections/sepsis. In this study 13 of 18 cases with a microbiologically related COD had elevated CRP values ($p < 0.05$). Considering that result and the literature on the subject [6,23], one would suggest that CRP measurement is carried out at the beginning of the autopsy. This would give an early indication of a possible infection in cases where antemortem information does

not suggest such, allowing for microbiological sampling before significant handling of the body and a hence reduced risk of mechanical contamination, both by direct transfer of bacteria and by displacement of bowel-near blood in arteries and veins.

Besides the possibility of improved sampling methods we also need to consider other methods to improve the microbiological testing in relation to autopsy. PCR analyses have been proved useful [24], and although it seems to be excellent with regards to virus investigations, it does not bypass the biggest pitfall in bacterial analysis, the postmortem spread. Since it is even more sensitive than culturing [8] it might possibly yield an even higher number of positive results. PCR analysis targeting specific bacterial toxins or the toxin-genes might be of some use [25], investigations into PM bacterial toxin production could be an interesting area for future work.

5. Conclusion

Microbiological sampling remains an important part of the autopsy yielding the cause of death in 42.8% of the cases in which it was performed. Optimized storage of the body and precautions to avoid contamination greatly enhance the value of the results.

The decision to classify a COD as microbiological rests on a combination of factors, including CRP levels, histology and an analysis of the microbiological agents.

A raised CRP level is correlated with microbiologically related COD and if performed at the beginning of the autopsy could be used as a guideline to initiate a microbiological autopsy, before mechanical manipulation leads to contamination.

Histology is an important part of the microbiological investigation in almost 2/3 of the cases with a microbiologically related COD, verifying inflammation in the cultured material, and in a few cases being the decisive factor, though supported by other information, in the diagnosis where the microbiological results were negative. Therefor all sites sampled for microbiological testing should also be histologically examined.

In very few of our cases only one single microbiological agent was found or an agent which is without doubt the sole cause of death. Several of the microorganisms contributing to our natural flora, colonizing human skin, respiratory tract or other inner surfaces or which are found in abundance in our surroundings can lead to life-threatening infections under the right circumstances, i.e. persons with underlying illness, immune suppression etc. Conversely widely found microorganisms known to cause sepsis could be the result of postmortem spread, considering their established virulence, target age group and point of infection. Investigators should always take into account all findings and anamnestic information in each case compared to the detected microorganisms before making any conclusions. Hence a definite list of “true pathogens” is nearly impossible to compile, and we suggest consulting microbiological literature on each microorganism, once it has been deemed of interest due to the above factors.

Acknowledgements

I would like to thank Marianne Kragh Thomsen, Consultant in clinical microbiology, Department of Clinical Microbiology, Aarhus University Hospital, for the help with microbiological nomenclature.

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