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Research Article

ATP measurement as method to monitor the quality of reprocessing flexible endoscopes

ATP-Bestimmung als Methode zur Qualitätskontrolle der Endoskopaufbereitung

Abstract

Insufficient performance of cleaning and disinfection of flexible endoscopes can pose an infection risk to patients. Actually quality of reprocessing is checked by performing microbiological cultures. Unfortunately, their results are not available on the same day so that more rapid methods are desirable. We compared the ATP (adenosine triphosphate) bioluminescence for hygiene checking of the reprocessing procedures of 108 flexible endoscopes with routine microbiological culture technics. Sensitivity and specifity of ATP bioluminescence was calculated. 28 endoscopes showed bacterial growth of at least one sample. Depending on the applied threshold of bioluminescence between 67 and 28 endoscopes were positive. Sensitivity varied between 0.46 and 0.75 and specifity between 0.43 and 0.81. ATP bioluminescence does not replace routine microbiologic methods but it can indicate the need of immediate check of reprocessing.

Zusammenfassung

Von unzureichender Reinigung und Desinfektion flexibler Endoskope können Infektionsrisiken für Patienten ausgehen. Die Endoskopaufbereitung wird derzeit durch mikrobiologische Untersuchungen kontrolliert. Der Nachteil ist, dass die Ergebnisse oft erst nach Tagen vorliegen, so dass ein Schnelltest wünschenswert wäre. Wir verglichen die ATP(Adenosintriphosphat)-Biolumineszenz-Bestimmung zur hygienischen Kontrolle der Endoskopaufbereitung mit konventionellen mikrobiologischen Kulturen bei 108 flexiblen Endoskopen und bestimmten die Sensitivität und Spezifität der ATP-Biolumineszenz. 28 Endoskope zeigten mindestens in einer mikrobiologischen Kultur Bakterienwachstum. Abhängig vom zugrunde gelegten Grenzwert der Biolumineszenz waren zwischen 67 und 28 der Endoskope bei der ATP-Bestimmung positiv. Die Sensitivität der ATP-Bestimmung lag zwischen 0,46 und 0,75, die Spezifität zwischen 0,43 und 0,81. Die ATP-Bestimmung ersetzt nicht die regelmäßigen mikrobiologischen Untersuchungen, sie kann jedoch die Notwendigkeit einer sofortigen Überprüfung der Aufbereitungsmodalitäten anzeigen.

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Introduction

Infections by endoscopes have been described as a consequence of insufficient cleaning and disinfection [1], [2]. Reports regard duodenoscopes [3], coloscopes [4], and bronchoscopes [5], [6] and concern exists regarding the possible transmission of infective agents like hepatitis B-/C- virus, HIV, Mycobacterium tuberculosis and Helicobacter pylori [7]. An investigation in southern Germany has shown that 50% of endoscopes were still contaminated by bacteria after reprocessing [8]. American [9], [10], [11] and European [7], [12], [13], [14], [15] recommendations for the reprocessing of endoscopes have been published. Whereas in Germany and various other countries control of cleaning and disinfection by microbiological methods is recommended the use of microbiological cultures to routinely check the reprocessing process is discussed controversely in USA [7], [16]. Unfortunately, there are many disadvantages of microbiological cultures: Getting results lasts for days, so the endoscopes are used with other patients. Additionally, viruses, Helicobacter and M. tuberculosis are not at all included and slowly growing organisms only if the time of incubation is long

enough. So a more rapid method for checking the reprocessing of endoscopes is needed.
ATP (adenosine triphosphate) measurement is used as indicator of cleaning control [17], [18] in food and kitchen hygiene. ATP is as well an indicator of organic as of microbiological contamination. It is a simple method measuring the amount of light which is emitted when the enzyme luciferase comes into contact with molecular ATP and which is directly proportional to the amount of ATP [19]. ATP measurement may be a suitable method to control the quality of endoscope reprocessing as it is measuring cleaning effectiveness which may indicate the reduction of infection risk.
We did an investigation to compare the ATP bioluminescence for hygiene checking of reprocessing with routine microbiological cultures.

Methods

Between January and December 2003 we examined 108 endoscopes (40 gastroscopes, 18 coloscopes, 8 duodenoscopes and 42 bronchoscopes) after reprocessing. Sterile swabs were moistened with sterile 0.9% NaCl. We took swabs of distal end and rinsing valve and rinsed the operating channel with 20 ml sterile 0.9% NaCl. We did not use neutralizers. Swabs and 0.5 ml of rinsing fluid were inoculated on blood agar, MacConkey agar and Sabouraud agar and incubated for 48 hours at 37° C and 7 days at 22° C respectively. Bacterial species identification followed routine microbial laboratory proceedings (API biomerieux). Every bacterial growth was considered microbiological positive regardless of species or number of cfu (colony forming unit). ATP and AMP (adenosine monophosphate) bioluminescence was determined using Lumitester PD 10 (Scil Diagnostics). The assay was carried out according to the manufacturer´s instructions immediately after swabbing. Reagent blanks were obtained using sterile swabs moistened with sterile 0.9% NaCl, instrument disinfectant and endoscope cleaner used for endoscope reprocessing. Bioluminescence readings were expressed as relative light units (RLU). Thresholds for bioluminescence were chosen between 30 and 100 RLU according to manufacturer's personal recommendation and after determining RLU of disinfectant and cleaner below 10 RLU. Sensitivity and specifity of ATP bioluminescence compared with microbiological culture as a gold standard were calculated for all types of examined endoscopes together and displayed in a ROC curve for various threshold values. The area under the ROC curve, which is usually chosen as the summary measure of diagnostic accuracy, was also computed.

Results

Results of microbiological culture and ATP bioluminescence are shown in Table 1. Microbiological cultures of 28 endoscopes (26%) showed bacterial growth. 13% of checked duodenoscopes, 28% of coloscopes, 23% of gastroscopes and 31% of bronchoscopes were bacterially contaminated. The detected organisms were Pseudomonas aeruginosa, other non fermenting gramnegative rods, Enterobacteriaceae, Staphylococcus aureus, koagulase negative Staphylococci, Corynebacteriae, Bacilli, Candida and moulds. Dependent on the chosen threshold between 28 (26%) and 67 (62%) endoscopes were positive for ATP bioluminescence. ATP bioluminescence of 75% of duodenoscopes, 67% of coloscopes, 63% of gastroscopes and 57% of bronchoscopes was above a threshold of 30 RLU. There were still 25% of duodenoscopes, 50% of coloscopes, 30% of gastroscopes and 24% of bronchoscopes above a threshold of 100 RLU. ATP bioluminescence of 5 bronchoscopes and 2 gastroscopes was below 30 RLU despite being microbiologically contaminated. Choosing 30 RLU as threshold 21 endoscopes (8 bronchoscopes, 7 gastroscopes, 5 coloscopes and 1 duodenoscope) were positive for ATP bioluminescence and microbiological culture and 34 endoscopes (13 bronchoscopes, 13 gastroscopes, 6 coloscopes and 2 duodenoscopes) were negative. 46 endoscopes (16 bronchoscopes, 18 gastroscopes, 7 coloscopes and 5 duodenoscopes) had an ATP bioluminescence above 30 RLU despite negative microbiological result. Above a threshold of 100 RLU 65 endoscopes (24 bronchoscopes, 23 gastroscopes, 12 coloscopes and 6 duodenoscopes) had concordant negative and 13 endoscopes (5 bronchoscopes, 4 gastroscopes, 3 coloscopes and 1 duodenoscope) concordant positive results. Microbiological cultures of 15 endoscopes (5 bronchoscopes, 8 gastroscopes, 1 coloscope and 1 duodenoscope) were negative despite ATP bioluminescence above 100 RLU. The ROC curve of sensitivity and specifity for thresholds between 30 and 100 RLU is presented in Figure 1. The area under the ROC curve is 0.63. Compared with microbiological culture as a gold standard sensitivity of bioluminescence varied between 0.75 for a threshold of 30 RLU (95% confidence interval 0.55-0.89) and 0.46 for 100 RLU (95% confidence interval 0.28-0.66) and specifity between 0.43 (95% confidence interval 0.32-0.54) and 0.81 (95% confidence interval 0.71-0.89) respectively (Table 2).

Discussion

In our study, 26% of tested endoscopes showed microbiological contamination. The number of contaminated endoscopes in our investigation is in accordance with the results of Moses and Lee [20], who found between 12% and 24% positive cultures during a 10-year study period. It is much lower than that of the HYGEA study [8]. Moses and Lee examined only endoscopes used in a clinical institution and reprocessed in an automated washer whereas in half of endoscopy facilities of the HYGEA study endoscopes were reprocessed manually. In our study 92 endoscopes (85%) were reprocessed in an automated washer.

Table 1: Number of endoscopes with a bioluminescence value above the threshold compared with microbiological culture

Kind of endoscope	Microbiological culture	Threshold (RLU)							
		>30	>40	>50	>60	>70	>80	>90	>100
bronchoscope n=42	positive n=13	8	5	5	5	5	5	5	5
	negative n=29	16	15	8	6	5	5	5	5
gastroscope n=40	positive n=9	7	5	5	5	5	5	4	4
	negative n=31	18	14	12	10	9	8	8	8
coloscope n=18	positive n=5	5	4	4	4	3	3	3	3
	negative n=13	7	4	4	3	3	2	2	1
duodenoscope n=8	positive n=1	1	1	1	1	1	1	1	1
	negative n=7	5	3	3	3	3	2	1	1
all endoscopes n=108	positive n=28	21	15	15	15	14	14	13	13
	negative n=80	46	36	27	22	20	17	16	15

Table 2: Sensitivity and specifity of bioluminescence as compared with microbiological culture and 95% confidence interval

Threshold (RLU)	Sensitivity (95% confidence interval)	Specifity (95% confidence interval)
30	0.75 (0.55-0.89)	0.43 (0.32-0.54)
40	0.54 (0.34-0.72)	0.55 (0.47-0.66)
50	0.54 (0.34-0.72)	0.66 (0.54-0.76)
60	0.54 (0.34-0.72)	0.73 (0.61-0.82)
70	0.50 (0.31-0.69)	0.75 (0.64-0.84)
80	0.50 (0.31-0.69)	0.79 (0.68-0.87)
90	0.46 (0.28-0.66)	0.80 (0.70-0.88)
100	0.46 (0.28-0.66)	0.81 (0.71-0.89)

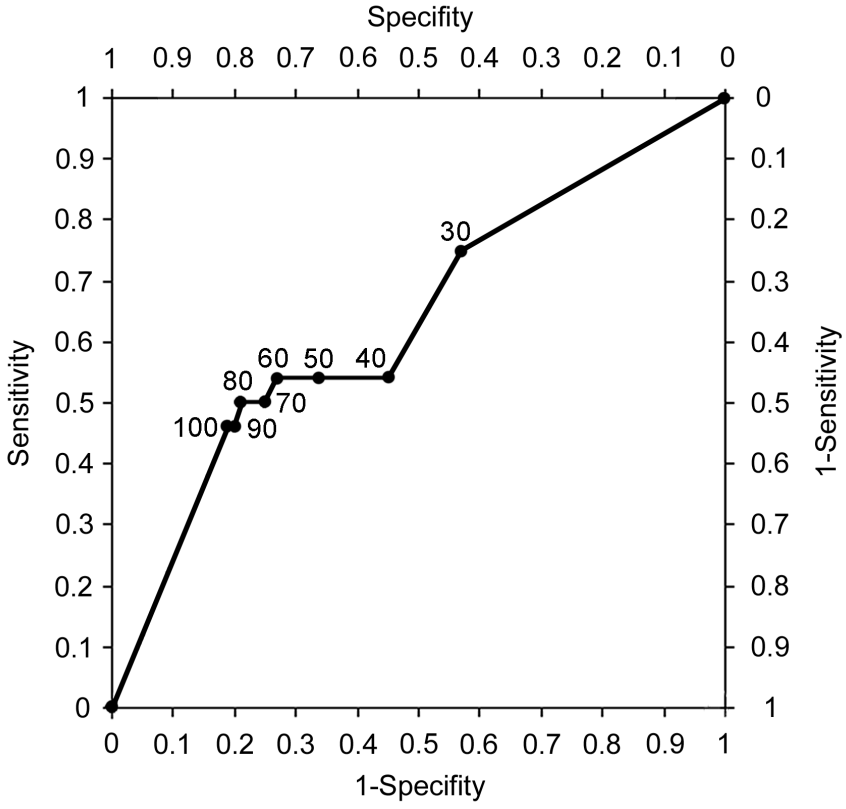

Figure 1: ROC curve of bioluminescence compared with microbiological culture as gold standard for thresholds between 30 and 100 RLU

Dependent on the chosen threshold between 62% and 26% of the tested endoscopes had a positive bioluminescence result indicating possible organic contamination. In order to calculate sensitivity and specifity of ATP bioluminescence there must be another method which truly indicates contamination of reprocessed endoscopes. The only established method for checking endoscope reprocessing is microbiological culture. Microbiological culture may fail in indicating all contaminated endoscopes. There may be non viable organisms or organisms which cannot be cultured on conventional culture medium and other than bacterial contaminations are possible. Because of the absence of other methods for checking endoscopes we calculated sensitivity and specifity of ATP bioluminescence compared to microbiological culture as gold standard. Sensitivity and specifity of bioluminescence differ dependent on the chosen threshold. In our study sensitivity was only 0.75 even when the chosen threshold of RLU was low. The ROC curve of ATP bioluminescence presented in Figure 1 with an area under the curve of 0.63 indicates that there is no strong concordance between ATP bioluminescence and microbiological culture. Our results are similar to those found by Murphy et al. [18] for testing food contact surfaces. Murphy et al. suspected that conventional microbiology is more sensitive than ATP bioluminescence when total ATP is low [18]. Bacterial ATP content may be below the limit of ATP bioluminescence. Different bacterial specimen can contain different amounts of ATP and the amount of ATP also depends on the metabolism of the organisms [19]. ATP bioluminescence may also be influenced by the number of viable bacteria present. We did not differ between kind of specimen and number of cfu cultured. The number of cfu found on most swabs was very low and this may explain the low sensitivity of ATP bioluminescence compared to routine microbiology in our study. Additionally the low specifity of ATP bioluminescence may be explained by the fact, that not only viable bacteria but also other organic contamination is detected. Similar to our study Poulis et al. [21] could not find a clear relationship between ATP bioluminescence measurements and number of cfu on surface plates under practical conditions on surfaces in a factory. Alfa et al. [22] reported that the presence of high residual soil (protein, carbohydrate, hemoglobin and endotoxin) did not correlate with microbiological contamination of reprocessed endoscopes. Thus measurable ATP bioluminescence may indicate contamination of endoscopes without presence of cultivable microorganisms. Reprocessed endoscopes should be clean. A clean endoscope should not only show a less amount of viable organisms but also a less amount of all organic contamination and ATP sources. The presence of any ATP source may indicate an infectious risk for consecutively examined patients and should be avoided irrespective of cultivable bacteria.

We conclude that ATP bioluminescence does not replace routine microbiologic methods but it should be applied additionally to check endoscope reprocessing. In contrast to microbiologic methods results of ATP bioluminescence are available at once and can indicate the need for checking the reprocessing practice immediately.

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