Implementation of an Acute Coronary Syndrome Simulation Training Strategy for Emergency Healthcare Professionals

Maíra Viana Rego Souza-Silva,1,2 Priscilla Fortes de Oliveira Passos,1,2 Thais Ribeiro Lemos,1,2 Bárbara Campos Abreu Marino,1,2 Thaitane Dantas Dias,2 Gelsiane Sousa Braga Machado,2 Edson Alexandre Silva Carvalho,1,2 Gabriel Almeida Silqueira Rocha,1,2 Antonio Luiz Ribeiro,1,2 Milena Soriano Marcolino1,2

Faculdade de Medicina, Universidade Federal de Minas Gerais,1 MG - Brazil
Centro de Telessaúde do Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG),2 MG - Brazil

Abstract

**Background:** The knowledge on the management of patients with acute coronary syndrome (ACS) is essential to reduce the gap between evidence and practice.

**Objective:** To describe a simulation training strategy for emergency healthcare professionals and provide preliminary data on knowledge acquisition, learners’ confidence and prescription of medications after training.

**Methods:** The training was part of the implementation of two myocardial infarction systems of care. It comprehended lectures and simulation-based learning using high and low-fidelity mannequins and actors. It was tested in two phases: the first one in Belo Horizonte and the second one in Montes Claros, both in the state of Minas Gerais. A test was applied before and after training to assess knowledge acquisition. Confidence to perform thrombolysis in ST-elevation myocardial infarction (STEMI) patients was assessed using a questionnaire, and the impact on medication prescription analyzed STEMI patients admitted to hospitals in Montes Claros.

**Results:** In the first phase, 156 professionals answered both tests: 70% of them improved their results and the median number of right answers increased (6, interquartile range [IQR] 5-7; vs 7 (IQR) 6-9; p < 0.05). In the second phase, 242 professionals answered both tests: 58% of the physicians and 83% of the nurses obtained better test scores. Participants referred a positive impact on their clinical practice, 95% reported feeling very secure when perform fibrinolysis after the training, and there was also an impact on medication prescription.

**Conclusions:** There was an impact on the learners’ knowledge acquisition and confidence using our two-phase training model, with evidence of impact on performance. (Int J Cardiovasc Sci. 2019;32(3)227-237)

**Keywords:** Acute Coronary Syndrome; Myocardial Infarction; Myocardial Reperfusion; Emergency Service Hospital; Quality Indicators, Health Care.

Introduction

The knowledge on the management of patients with acute coronary syndromes (ACS) is essential to any emergency healthcare provider. This condition is highly prevalent globally and potentially hazardous, responsible for 31% of all deaths in 2015.1,2 It is also known that mortality is income-related and at least three quarters of deaths occur in low-and middle-income countries (LMICs), such as Brazil.3 In this country, ACS is an important cause of hospitalization and the leading cause of mortality, accountable for almost 28% of deaths in 2014.4 5,6 The gap between research knowledge and guideline recommendations to their utilization in clinical practice impacts on the quality of care delivered and clinical outcomes.5,6 Therefore, it is necessary to provide means to keep staff updated on ACS guidelines aiming to enhance performance and, ultimately, to improve patient care.
Simulation-based techniques are well-established means to improve both individual and teamwork performance by increasing awareness of current protocols, development of practical skills and enhancing clinical reasoning.7-9 Issenberg et al.,10 reviewed studies to evaluate the features of simulation techniques on medical education and observed that it is an effective learning technique that complements bedside medical education.10 However, studies evaluating the use of simulation education in LMICs are limited. There is a lack of permanent education programs and restrained funds to invest in simulations.11 We hypothesize that using simulations in LMICs can improve the healthcare team’s performance and knowledge on ACS, leading to a more effective care to be delivered to the patients.12,13

Thus, the purpose of this study is to evaluate the implementation of a two-phased, large-scale ACS training program across multiple learners in resource-limited areas. Furthermore, we provide preliminary data on knowledge acquisition, learner confidence, and impact on medication prescription after the strategy to foster further use of the program.

Methods

Participants

Physicians and nurses from public hospitals, emergency care units (Unidades de Pronto Atendimento, UPA) and the ambulance service (Serviço de Atendimento Médico de Urgência, SAMU) of Belo Horizonte and 89 municipalities in the north of Minas Gerais state, Brazil, were invited to join the training, focusing mainly on professionals responsible for the care of patients with ACS. Also, professionals from institutions belonging to the Telehealth Network of Minas Gerais (TNMG) were also invited to participate.

The TNMG is a large public telehealth service launched in 2005 by seven public universities in Minas Gerais state. At first, TNMG focused on telecardiology and assisted primary care settings of remote municipalities in Minas Gerais state, Brazil. The initiative has expanded to other municipalities (covering 813 of the 853 municipalities of the state) and also to emergency services.14,15 Furthermore, the service participated in the development of the acute myocardial infarction (AMI) system of care in Belo Horizonte and in the north of Minas Gerais, and this training was part of the implementation of those care systems.16,17

Intervention design

The training consisted of four sections: (1) pre-assessment, (2) lectures, (3) simulation-based stations and (4) post-assessment.

Firstly, the participants’ previous knowledge on the subject was assessed through a pre-test, which consisted of 10 multiple-choice questions related to diagnosis, management and treatment of ACS patients based on standard protocols. Afterwards, they had lectures about those topics, based on current ACS protocols.18-24 At the end of the section, the lecturer dedicated a moment to listen to and answer queries from the audience. Then, they participated in a simulated environment to perform what they had learned in a practical setting. Lastly, they completed the same test taken at the beginning of the training. The goal was to determine knowledge acquisition.

Simulation setting

The simulation-based session comprehended five stations to practice the following situations: (1) ST-elevation myocardial infarction (STEMI) with thrombolysis indication, (2) STEMI with indication of referral for primary angioplasty, (3) AMI with cardiogenic shock and respiratory failure, (4) Non-ST-elevation myocardial infarction (NSTEMI) and (5) Recording and transmitting the digital electrocardiogram. Each situation was reproduced in a simulated scenario guided in real-time by a facilitator. The simulation stations used one high-fidelity mannequin, one low-fidelity mannequin and three actors simulating patients (Figures 1 and 2).

The facilitator followed a simulation plan that included: 1) primary and secondary objectives of each station; 2) case description; 3) response plan to the simulator depending on the participants’ possible actions; 4) scripts for the actors responsible for voice simulator and other participants; and 5) a debriefing plan. All scenarios were previously tested.

In each station, two individuals created the learning environment and 6-7 others watched the two first ones. The simulation followed the consecutive sequence: (i) participants were welcomed by the facilitator; (ii) explanation of basic simulation techniques; (iii) introduction of the simulator and simulation environment; (iv) separation in two groups: “hot seats” and observers (v) introduction of the scenario; (vi) performance of the scenario; (vii) facilitated debriefing, stage in which the facilitator mediated constructive feedback
Figure 1 - Scenario in which participants can practice recording and transmitting the digital electrocardiogram, using an actor, simulating a non-ST elevation myocardial infarction.

Figure 2 - Instructor performing introduction of the simulator and simulation environment in the station about ST-elevation myocardial infarction with indication of thrombolysis, using a high-fidelity mannequin.
in order to stimulate critical reflection about the simulation; (viii) summarizing of the most important topics.25

**First phase**

This phase was developed in Belo Horizonte, Brazil. Physicians and nurses from emergency care units and SAMU were invited to join the training, focusing mainly on professionals responsible for the care of ACS patients.

**Second phase**

This phase was developed in Montes Claros, Brazil. It was part of a quasi-experimental study, Minas Telecardio 2 project, a three-phase study (baseline, implementation and post-implementation phases) which assessed the impact of the implementation of the myocardial infarction care system in the north of Minas Gerais state. The training reported here was part of the implementation phase.17,26

In this region, the health system is divided into nine micro-regions, covering 89 municipalities in an area consisting of 128,000 km² and comprising a population of 1.6 million inhabitants. There are 18 public hospitals in the area, but 9 of those are concentrated in Montes Claros, the main municipality of the region. SAMU has 47 ambulances (7 with doctors and 40 with nurse technicians).26

There were two training sessions. The first one, on April 2014, was carried out in Montes Claros, and physicians and nurses from emergency services of the 18 public hospitals and ambulance services (SAMU) of the north region of Minas Gerais were invited to participate.

The model and duration of the training were similar to the first phase, except for the test applied to nurses, which included specific questions about their role in the management of ACS patients. All professionals were invited to answer a feedback survey. It included questions about the participant’s clinical practice, how often they had managed chest pain in the last month and how secure they felt when performing thrombolysis (when indicated) after the course.

The second training session was carried out in August/September 2014. This one took place in five different municipalities distributed throughout the region, to make it easier for professionals who lived far away from Montes Claros to participate. Physicians and nurses from the emergency services of the 18 public hospitals and ambulance services (SAMU) of the north region of Minas Gerais were invited to participate. For this one, no pre and post-tests were applied.

Data about medication prescription was collected in three different moments: baseline (June 19th, 2013 to March 31st, 2014); post-implementation phase, just after the training sessions happened (September 1st, 2014 to May 31st, 2015); and eight months after the end of the Minas Telecardio 2 project (June 1st, 2015 to January 31st, 2016). Data on the prescription of aspirin, P2Y12 inhibitor, heparin and statin medication within the first 24 hours after admission and at discharge were collected from STEMI patients admitted to hospitals in Montes Claros.

**Statistical analysis**

The data were analyzed using the program Statistical Package for the Social Sciences, version 20.0 (SPSS Inc, Chicago, IL, EUA). The evaluation of the distribution of continuous variables was performed using the Kolmogorov-Smirnov test. The continuous variables were expressed as mean and standard deviation or median and interquartile range, as appropriate, while categorical variables were expressed as absolute number and percentage. The test questions were classified into different categories depending on the subject: diagnosis, treatment or both. This classification was used to analyze the results of both tests stratified by the subject. The results of the pre- and post-tests were compared using McNemar test. In order to evaluate the influence of workplace and years since graduation on the participants’ performance in the test, the non-parametric Spearman’s correlation coefficient was calculated. The p value considered significant was set at 0.05.

**Results**

**First phase**

A total of 189 professionals participated in the training, of which 86 were doctors and 103 nurses. Most were females (68.0%), and there was a predominance of professionals from the emergency care units (50.8%), hospitals (29.5%) and ambulance services (17.2%). Additionally, 18.9% of them had graduated up to 1 year before, 24.6% had graduated 2 to 5 years before, 16.4% had graduated 6 to 10 years before and 38.5% had been working for more than ten years in the profession.

One hundred and fifty-six professionals answered both tests, which corresponds to 82.5% of the total (Figure 3). In the comparative data analysis, it was observed an improvement in the participants’ performance in the post-test, since 66.0% of the professionals achieved better scores.
To highlight the improvement in the professionals’ knowledge acquisition, a comparative analysis of the total number of correct answers in both tests was performed and is shown in Table 1. An increase in the number of correct answers was observed in the post-test when compared to pre-test, with a median of 6 right answers in the pre-test (interquartile range [IQR] 5-7) and 7 in the post-test (IQR 6-9) (p < 0.0001). In addition, the lowest score comprised two correct answers to questions in the pre-test and increased to four in the post-test. Based on the type of question, an individual analysis showed an increase in the number of correct answers in 8 out of 10 questions (p < 0.05). As for the remaining questions, in one of them the number of right answers in the pre-test was high (97.4%) and remained high in the post-test results (99.4%). In the last question, the number of correct answers remained low in both tests, as shown in Table 2. No correlation was observed between years of work experience (Spearman -0.058, p = 0.53) or workplace (Spearman -0.034, p = 0.71) and knowledge acquisition. These results were similar when assessing only medical doctors (occupation time Spearman’s = -0.041, p = 0.75; workplace Spearman’s = -0.10 p = 0.42).

**Second phase**

A total of 252 professionals participated in the first training session, of which 81 were doctors and 171 nurses. Two-hundred and forty-two professionals (75 doctors and 167 nurses) answered both tests, which corresponded to 96% of the total (Figure 3). The profile analysis of physicians (n = 75) revealed that most were males (76.0%). There was a predominance of the ambulance service professionals (51.0%), followed by hospital employees (45.0%) and 21.0% had graduated up to one year before, 41.0% had graduated 2 to 5 years before, 20.0% had graduated 6 to 10 years before and 17.0% had been working for more than ten years in the profession. As for nurses, the analysis of their profile (n = 167) indicated that most were women (58.0%), with a predominance of professionals working in hospitals (73.1%) followed by those working in ambulance services (25.7%). Ten percent had graduated up to one year before, 42.0% had graduated 2 to 5 years before, 42.0% had graduated 6 to 10 years before and 5.0% had been working in the profession for more than ten years.

In the comparative data analysis shown in Table 1, an improvement in the results was observed in the post-test, since 78.0% of the sample (58.0% of physicians and 83.0% of nurses) obtained better results. Medical doctors reached a median of 7 correct answers in the pre-test (interquartile range [IQR] 2-8) and 8 in the post-test (IQR 7-9) (p < 0.0001). Nurses had a median of 6 right answers in the pre-test (interquartile range [IQR] 4-7) and 9 in the post-test (IQR 7-9) (p < 0.0001). Furthermore, as shown in Table 2, the lowest score for physicians was two questions in pre-test and increased to four in post-
Table 1 - Number of right answers by the participants (n = 156) in the first phase, and physicians (n = 75) and nurses (n = 167) in the second phase

<table>
<thead>
<tr>
<th>Right answers</th>
<th>First phase</th>
<th>Second phase</th>
<th>Physicians</th>
<th>Nurses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2 (1.3)</td>
<td>-</td>
<td>1 (1.4)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>11 (7.1)</td>
<td>-</td>
<td>1 (1.4)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>14 (9.0)</td>
<td>6 (3.8)</td>
<td>4 (5.3)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>5</td>
<td>37 (23.7)</td>
<td>20 (12.8)</td>
<td>10 (13.3)</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>6</td>
<td>32 (20.5)</td>
<td>32 (20.5)</td>
<td>18 (24.0)</td>
<td>11 (14.7)</td>
</tr>
<tr>
<td>7</td>
<td>30 (19.2)</td>
<td>23 (14.7)</td>
<td>18 (24.0)</td>
<td>19 (25.3)</td>
</tr>
<tr>
<td>8</td>
<td>15 (9.6)</td>
<td>32 (20.5)</td>
<td>17 (22.6)</td>
<td>10 (13.3)</td>
</tr>
<tr>
<td>9</td>
<td>12 (7.7)</td>
<td>23 (14.7)</td>
<td>6 (8.0)</td>
<td>19 (25.3)</td>
</tr>
<tr>
<td>10</td>
<td>3 (1.9)</td>
<td>20 (12.8)</td>
<td>-</td>
<td>10 (13.3)</td>
</tr>
</tbody>
</table>

Table 2 - Comparative analysis of the number of right answers per question (n = 156) in the first phase

<table>
<thead>
<tr>
<th>Type of question</th>
<th>Pre-test</th>
<th>Pos-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>1 Diagnosis</td>
<td>152 (97.4)</td>
<td>155 (99.4)</td>
<td>0.250</td>
</tr>
<tr>
<td>2 Therapy</td>
<td>68 (43.6)</td>
<td>84 (53.8)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>3 Therapy</td>
<td>90 (57.7)</td>
<td>126 (80.8)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>4 Diagnosis and Therapy</td>
<td>104 (66.7)</td>
<td>132 (84.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>5 Diagnosis and Therapy</td>
<td>118 (75.6)</td>
<td>108 (69.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>6 Diagnosis and Therapy</td>
<td>103 (66.0)</td>
<td>134 (85.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>7 Therapy</td>
<td>72 (46.2)</td>
<td>104 (66.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>8 Diagnosis and Therapy</td>
<td>61 (39.1)</td>
<td>100 (64.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>9 Therapy</td>
<td>61 (39.1)</td>
<td>58 (37.2)</td>
<td>0.250</td>
</tr>
<tr>
<td>10 Diagnosis</td>
<td>109 (69.9)</td>
<td>139 (89.1)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

In this study, a training strategy for ACS management using simulation techniques for emergency healthcare professionals improved knowledge acquisition, test and for nurses it went from zero to five questions. Table 3 shows the individual analysis of each question, for physicians and nurses.

One-hundred and thirty-seven healthcare professionals filled out the survey, of which 58 were nurses (29.9% of the total) and 76 were physicians (93.8% of the total). Most of the sample (52.6%) had had less than 5 years of work experience since graduation. A total of 58 physicians (76.3% of the respondents) informed having treated at least five patients with chest pain during the last month, while 26 (34.2%) reported having treated 5 to 10 patients and 32 (42.1%) professionals treated over 10 patients. After the training, 128 professionals (95.5%) reported feeling “very secure” when performing fibrinolysis.

A total of 24 physicians and 119 nurses participated in the second training session.

Regarding the impact on medication prescription, the results are shown in Table 4.

Discussion

In this study, a training strategy for ACS management using simulation techniques for emergency healthcare professionals improved knowledge acquisition,
measured by pre- and post-test assessments in two different scenarios of AMI care system implementation. It provided evidence of an increase in physicians’ confidence to perform thrombolysis in STEMI patients, and an improvement in medication prescription within the first 24 hours after admission and at discharge.
In both phases, the performance of healthcare practitioners in the post-test was better than in the pre-test. More than 66.0% of the professionals achieved better results in the post-test in the first phase and 78.0% in the second phase. There was a significant improvement in 8 out of the 10 questions with statistical significance (p < 0.05) in the first phase. It shows that, after training, professionals had an increase in the theoretical domain regarding managing ACS patients, which contributes to better use of these lessons in clinical practice.

In the second phase, the number of right answers increased for physicians and nurses, but nurses achieved greater improvement. Nurses improved their performance in all questions with statistical significance and the median number of correct answers increased significantly. Meanwhile, physicians improved their performance in 9 out of 10 questions, yet there was no statistically significant difference in 5 of them, due to sample size limitations (it was not possible to apply the McNemar Test in 2 questions, as the entire sample answered correctly in the post-test). As it was evident when looking at the pre-test results, medical doctors tended to have better previous knowledge about ACS than nurses. Considering that, we hypothesize that the training was especially valuable for professionals with less previous knowledge on the subject. As for medical doctors, a huge disparity in medical education was also evident, as the result of pre-test varied significantly among participants: the pre-test IQR of correct answers was 2-9 in the pre-test and decreased to 7-9 in the post-test.

The training also impacted on the participants’ self-confidence, as 95% reported feeling very secure when performing fibrinolysis after the training. Even though participants did not have previous experience with simulation during graduation, they accepted and adapted well to the training model. They participated in the simulated scenarios, debriefing and in the feedback sessions.

This simulation experience had strengths and barriers. A specific characteristic that contributed to the success of our training was the fact that physicians and nurses were trained together. It was based on the idea that effective collaboration between these professionals can lead to a reduction in morbidity and mortality, fewer medical errors and enhance job satisfaction.27 Recently, training models have been recognized as important tools to improve teamwork and communication skills across the healthcare staff.28,29 Thus, we developed a framework that emphasized teamwork and communication. In each station, two participants (one physician and one nurse) worked together to respond to the clinical scenario and all participants were engaged in the debriefing. Furthermore, our training was also helpful to increase awareness of current guidelines. As Sussman et al.,30 points out, lack of familiarity of practitioners with the presenting literature is one of the causes leading to the bridge between research and clinical routine.30 For instance, the use of pre-hospital electrocardiogram (ECG) to diagnose and manage patients is accepted in the literature as an effective method to reduce morbidity and mortality of ACS.24,31 Nevertheless, this recommendation is not always followed in clinical practice.32,33 This divergence between scientific knowledge and its implementation reinforces the importance of strategies to increase awareness of the guidelines and their application.

On the other hand, despite the common use of simulation in high-income countries (HIC), its use in LMICs are still scarce.11,35 In these countries, restrained funding remains an obstacle to the implementation of simulation training programs.11 Building a simulation center involves the acquisition of equipment, trained personnel and adequate facilities, thus the costs are high.36 In our experience, funding was a great barrier to turn this program into a continued education program. The professionals underwent a similar training session for only one additional time and the program is current on hold due to lack of resources. Looking at successful simulation trainings in LMICs, they usually involve a collaborative network between various departments and institutions and we believed that it could be an alternative to our scarcity of funding.11 As for other barriers, it is important to mention the lack of employers’ support, in the sense that the hours healthcare professionals spent on training were not paid. In the second phase, we can highlight the distance from the workplace to the simulation center, which increased transportation costs and precluded the participation of physicians and nurses who lived far from the city where the training was being held (Montes Claros).

This experience was part of the implementation of the AMI care system in Belo Horizonte and in the northern region of Minas Gerais state. This initiative encompassed a multifactorial intervention across multiple institutions to improve the quality of treatment, which also included tele-electrocardiogram implementation and AMI care reorganization. Previous studies concluded that to achieve success in the implementation of care systems, the action plan has to be defined not only considering established guidelines, but also taking into consideration...
the regional differences. Several studies performed in developing countries highlighted the existence of obstacles related to poor infrastructure to the establishment of a care system. In those situations, the training strategy might be even more important, since any improvement in health represents significant benefits in patient care. Although using the same test to evaluate the participants makes it difficult to appraise the influence of the pre-test on the post-test performance, the increase in medication prescription within the first 24 hours after admission and at discharge is an evidence of the impact of the training strategy. The fact that the benefit decreased after time following the training (the prescription increased in the nine months after the two training sessions but decreased after this period) showed the importance of continued education for healthcare professionals. Due to the high turnover of emergency professionals, training sessions need to be repeated periodically to increase effectiveness.

The use of simulation-based education has increased gradually in recent years and it has been considered an important method to improve professional development, patient safety and to enable team work practice. It is a student-centered learning process that enables students to build their own knowledge from mistakes. A basic assumption is that learning from mistakes in a simulated environment can reduce the occurrence of errors in clinical practice and increase the practice of the correct attitudes. In a systematic review by Issenberg et al., debriefing was considered the most important step to determine effectiveness in simulation-based learning. We considered that the use of these principles had an important role for our training success. The feedback from the health professionals showed a positive impact on their daily tasks, as most participants submitted to the training stated that the strategy added confidence and safety to their clinical practice.

Corroborating our findings, the BRIDGE-ACS study (Brazilian Intervention to Increase Evidence Usage in Acute Coronary Syndromes) performed in Brazilian public hospitals, demonstrated the effectiveness of a multifactorial intervention to improve quality of treatment. This intervention included the use of reminders, checklists, distance and presential training using simulation techniques. There was a significant increase in the proportion of patients who received the recommended therapies (aspirin, P2Y12 inhibitors, anticoagulants and statins) within 24 hours after admission and at discharge. Thus, there is evidence of an impact on the increase in adherence to the ACS guidelines. Another gain that is not possible to be measured is the impact on physicians' and nurses' motivation, which was very important for the success of the MI care system.

The study has limitations. Knowledge acquisition was assessed immediately after training. We tried to assess the long-term impact of the training by collecting data on medication prescription (and there was no other intervention to improve it), but further studies should perform a thorough assessment of the impact on clinical outcomes.

**Conclusion**

This study showed how a theoretical educational intervention, associated with simulation-based training strategy, in the context of the implementation of MI care systems in resource-limited areas, showed an impact on learners’ knowledge acquisition in two different scenarios, with evidence of impact on the health providers’ confidence in performing thrombolysis in STEMI patients and medication prescription within 24 hours after admission and at discharge. These experiences can be used to guide future programs that can be expanded to the rest of the country.

**Author contributions**

Conception and design of the research: Marcolino MS, Ribeiro AL; acquisition of data: Dias TD, Machado GSB, Carvalho EAS, Rocha GAS, Marino BCA; analysis and interpretation of the data: Marcolino MS, Souza-Silva MVR, Passos PFOP, Lemos TR, Carvalho EAS, Rocha GAS; writing of the manuscript: Marcolino MS, Souza-Silva MVR, Passos PFOP, Lemos TR; and all authors participated in critical revision of the manuscript for intellectual content.

**Potential Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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Study Association

This article is part of the PHD thesis submitted by Bárbara Campos Abreu Marino, from Program in Infectious Diseases and Tropical Medicine (PG-IMT) at the Medical School of the Universidade Federal de Minas Gerais.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais under the protocol number 260/09. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent (and permission to use images) was obtained from all participants included in the study.

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