Prevalence of central venous catheter salvage in newborn with staphylococcal bloodstream infection

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ABSTRACT

Objectives: to establish the prevalence of salvage of central venous catheters in newborns with bloodstream infection caused by coagulase-negative staphylococci. Methods: retrospective cross-sectional study with 136 newborns admitted to the Neonatal Intensive Care Unit between 2011 and 2017. The total of 143 infection events undergoing antibiotic therapy were evaluated. Results: among the 143 infection events, 39 catheters in which antibiotic therapy was used were saved and in 69 cases, the device was removed. Positive central blood culture and single lumen catheter were factors associated with salvage failure. The probability of salvage decreased with infections diagnosed from 15 days of using the catheter. Negative blood culture raised the chance of salvage by fourfold. Conclusions: the use of antibiotic therapy in the treatment of infections resulted in a low prevalence of salvage of the central venous catheter. The probability of salvage was associated with variables of the device.

Descriptors: Newborn; Central Venous Catheterization; Catheter-Related Infections; Salvage Therapy; Staphylococcal Infections.

RESUMO


RESUMEN

Objetivos: establecer la prevalencia de rescate de catéteres venosos centrales en recién nacidos con infección del torrente sanguíneo ocasionada por estafilococos coagulasa-negativos. Métodos: estudio transversal retrospectivo con 136 recién nacidos ingresados en la Unidad de Cuidados Intensivos Neonatales, entre 2011 y 2017. Se evaluaron 143 eventos de infección sometidos a terapia antibiótica. Resultados: de los 143 eventos de infección, 39 catéteres en los que se utilizó antibioticoterapia fueron salvos y en 69 casos, se retiró el dispositivo. El hemocultivo central positivo y el catéter de un lumen fueron factores asociados con el fracaso del rescate. La probabilidad de rescate disminuyó con las infecciones diagnosticadas a partir de los 15 días de uso del catéter. El hemocultivo negativo multiplicó por cuatro la posibilidad de recuperación. Conclusiones: el uso de terapia antibiótica en el tratamiento de infecciones resultó en una baja prevalencia de rescate del catéter venoso central. La probabilidad de rescate se asoció con las variables del dispositivo. Descriptores: Recién Nacido; Cateterismo Venoso Central; Infecciones Relacionadas con Catéteres; Terapia Recuperativa; Infecciones Estafilocócicas.
INTRODUCTION

Neonatal care procedures in intensive care units (ICUs) require the use of advanced technology and the central venous catheter (CVC) is one of the most common invasive procedures used in these patients(16). We highlight the umbilical catheterization, the peripherally inserted central venous catheter (PICC) and phlebotomy(22). Together, these three types of catheterization are known as CVC(1). Vascular access through these devices is crucial for the survival of newborns (NB), providing safe intravenous therapy(14-15).

Umbilical catheterization is defined as the introduction of a catheter in the umbilical artery and/or vein(19). On the other hand, the PICC is an intravenous device inserted through a superficial or deep vein of the extremity that progresses to the distal third of the superior vena cava. PICC variations include 20-65 cm lengths, calibers from 1 to 6 French, one to three lumens, made of silicone, polyethylene, polyurethane or carbonate. The PICC is inserted by percutaneous puncture through metallic or plastic bipartite needles for later disposal(19). Phlebotomy consists of dissection, isolation, catheterization and ligation of the distal vein in most cases, although it is indicated in emergencies, when peripheral venous access cannot be obtained promptly(19).

The innumerable advantages arising from the implementation of CVC are indisputable. However, there may be complications, such as infectious ones, associated with unfavorable health outcomes(16). One of the most frequent complications is the catheter-related bloodstream infection (CRBSI)(19-21). The increased susceptibility of NB to infections is related to deficiencies of the immune system and the fragility of cutaneous and mucous barriers, therefore, this population has characteristics not observed in any group of patients at different periods of life(12).

Despite the several recommendations for reducing the number of CRBSI, its incidence is still high, being the most prevalent infection in the neonatal ICU (NICU)(13-16). It is often necessary to maintain the CVCs, especially in newborns, as their permanence may be the only access available for the administration of essential components for the survival of premature infants(17).

The salvage of central lines is limited to the maintenance of infected intravenous devices submitted to local and/or systemic antimicrobial therapy, indicated in stable patients with CRBSI, after the isolation of low virulent germs(18). The discussion about the salvage of these CVCs in neonatal care is not yet a common habit, and NICUs rarely have well-designed protocols on the treatment of devices after the diagnosis of CRBSI. Although neonatologists predominantly choose to replace the central line soon after the diagnosis of CRBSI, the literature offers some recommendations on this dilemma between maintenance or removal. Many guidelines advise maintaining and treating catheters infected with coagulase-negative staphylococci (CNS)(19-21).

OBJECTIVES

To establish the prevalence of salvage of central venous catheters in newborns with bloodstream infection caused by coagulase-negative staphylococci.

METHODS

Ethical aspects

This study was approved by the Research Ethics Committee of the Complexo Hospital de Clínicas of the Universidade Federal do Paraná and all guidelines that regulate research involving human beings were followed.

Study design, period and location

Cross-sectional observational study with retrospective data collection guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) tool(20). The study was conducted in the Postgraduate Program in Child and Adolescent Health at the Universidade Federal do Paraná and data were collected from medical records at the Hospital Infection Control Center and at the Medical and Statistics Archive Service of the Complexo Hospital de Clínicas of the Universidade Federal do Paraná. The data collection period was between November 2016 and May 2018.

Population or sample

The sample consisted of 136 newborns admitted to the NICU, in which 143 CRBSI events with the use of antibiotic therapy were studied, aiming at salvage of the CVC. In seven NB, two events were recorded, that is, these seven patients experienced two infection episodes associated with the same CVC.

Inclusion and exclusion criteria

The inclusion criteria for reviewing medical records were: episodes of bloodstream infection identified in NB using PICC or phlebotomy admitted to the NICU; cases confirmed in the laboratory with a positive blood culture result for CNS related to these devices, according to criteria provided in the literature and all CVCs submitted to antibiotic salvage therapy. Umbilical CRBSI were excluded, since salvage therapy in this type of CVC is not recommended by the literature(21-22), as well as cases without laboratory evidence, bloodstream infection by Gram-negative microorganisms and fungi, and bloodstream infections in NB diagnosed with primary immunodeficiency.

Study protocol

Information of interest was obtained from the Hospital Infection Control Center for the selection of patients who developed some type of infection between years 2011 and 2017. From this survey, only the cases of CRBSIs were selected.

After applying the eligibility criteria, the second step of data collection took place at the Medical and Statistics Archive Service in the months of March, April and May 2017, where the records of 136 patients were accessed and all relevant information was transcribed to the data collection instrument. This instrument included variables associated with newborns' antenatal history (medical diagnoses, type and causes of labor, maternal infection); perinatal variables (birth weight, sex, gestational age); and additional information based on potential risk factors for CRBSI.
described in the literature (length of hospital stay, use of total parenteral nutrition -TPN, fasting time, previous exposure to systemic antibiotic therapy and invasive procedures). The variables related to the CVC were: type (phlebotomy/PICC), number of access routes (one - single lumen; two - double lumen), caliber, length of stay and place of insertion. Finally, variables associated with the diagnosis of CRBSI and outcome of the infection event were investigated: presence of dysthermia, desaturation, food intolerance, apnea, ventilatory support, alteration of capillary glycermia, hemodynamic instability, bradycardia; complementary exams, such as infectious blood count (yes/no) and C-Reactive Protein (CRP) (positive/negative); central and peripheral blood culture (positive/negative); removal of the device by CRBSI (yes/no); antibiotic CVC salvage therapy (oxacillin/amikacin; vancomycin; teicoplanin); salvage of the CVC (yes/no); death related to CRBSI (yes/no).

Analysis of results and statistics

All collected data were recorded exclusively by the researcher in the instrument immediately after collection from the medical records. Subsequently, they were typed in an electronic spreadsheet, checked and exported to the statistical software (Statsoft®).

The measures of central tendency and dispersion were expressed as means and standard deviation (mean + SD) for continuous variables with symmetric distribution and in median, minimum and maximum values (median, minimum - maximum), for those with asymmetric distribution. Categorical variables were expressed by absolute and relative frequencies.

The estimation of the difference of continuous variables with normal distribution was performed by the parametric Student's t test, while for variables of asymmetric distribution, the non-parametric Mann-Whitney test was used. The estimate of difference between categorical variables was performed using Pearson's chi-square tests with Yates correction for 2x2 contingency tables and Pearson's chi-square tests for the other tables.

The Kaplan-Meyer Curve was constructed to estimate the accumulated probability of the catheter salvage according to the time of occurrence of the infection. The Multivariate Logistic Regression model was used to identify predictive variables for the outcome of venous catheter salvage. For all tests, a minimum significance level of 5% and a minimum testing power of 90% were considered.

RESULTS

Profile of the newborns studied

Seventy-four NB were female (54.4%), 61 male (44.9%) and one had ambiguous genitalia (0.7%), with a median birth weight of 1052.5 g (465.0-4325.0). The mean gestational age (GA) was 30.6±5.0 weeks, ranging from 22 to 41 weeks. Prematurity was observed in 110 cases (80.9%).

Cesarean delivery was performed in 83 cases (61.0%). The main causes of prematurity were pregnancy specific hypertensive disorder (23.7%) and prolonged rupture of amniotic membranes (18.5%). The cause of premature birth was not identified in 59 cases (43.7%). Malformations were observed in 38 NB (27.9%) and respiratory distress syndrome (RDS) in 34 cases (25.0%).

Most newborns received TPN (93.3%) and antibiotic therapy (93.4%) prior to salvage of the catheter. Previous invasive procedures (surgery, umbilical, venous and bladder catheterization, chest drainage, tracheal intubation) were observed in 60 newborns (44.1%).

Of the 136 catheters implanted, 120 were PICC (88.2%), of which 116 single lumen (90.6%), 1.9 (61.5%) or 2 (18.9%) French caliber. The median length of stay of the catheter was 18 days (1-64) and the catheter infection occurred, in median, with 10.5 days (2-39).

The median length of hospital stay was 69 days (4-200). Of the 136 NBs, 15 (11.0%) died, and five of these deaths were related to CRBSI (3.7%).

Variables related to the diagnosis of infection

The main signs of infection observed were desaturation (41.2%), worsening of respiratory distress (35.7%), food intolerance (35.0%), dysthermia (34.5%), hemodynamic instability (26.6%) and apnea (23.8%).

The blood count indicated signs of infection in 126 cases (88.1%) and CRP was high in 108 (75.5%) events. In all cases, blood culture was positive; in six cases (4.2%) it was positive only in central blood culture, in 81 (56.6%) only in peripheral, and in 55 events (39.2%) it was positive in both.

Central venous catheter salvage

Of the 143 CRBSI events, the CVC was removed in 104 (72.7%) events and saved in 39 (27.3%) events. The main cause of removal was infection (66.3%), followed by extravasation (12.5%), externalization (6.7%), obstruction (5.8%), death (5.8%) and catheter breakage (2.9%).

Considering the 143 events of CRBSI, patients were divided into two groups, according to whether or not the CVC was saved through antibiotic therapy.

- Group of CVC salvage (n = 39)
- Group of no CVC salvage (n = 69)

Table 1 shows the salvage therapies used. In both groups, the three regimens were used in similar proportions, so there was no difference in the distribution of antibiotics in the compared groups (p = 0.81). Consequently, no therapeutic regimen was more effective than the other for the catheter salvage.

Among the events in which catheter salvage did not happen, was observed a higher frequency of positive central blood culture (60.9% vs 28.2%; p <0.01) and a lower frequency of double lumen CVC (7.6% vs 21.1%) with a borderline significance level (p = 0.09). The length of stay of the CVC was evidently shorter in the no CVC salvage group (p <0.001) (Table 2).

The probability of stay of the CVC according to the time of occurrence of the infection was determined by the Kaplan-Meier survival analysis (Figure 1). The probability of catheter salvage decreased significantly with infections occurring from 15 days of catheterization.
Table 1 – Antibiotics used in salvage of infected catheters, Curitiba, Paraná, Brazil, 2011-2017

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oxacillin and amikacin</th>
<th>Teicoplanin</th>
<th>Vancomycin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No salvage (n = 69)</td>
<td>19 (30.2%)</td>
<td>25 (39.7%)</td>
<td>19 (30.2%)</td>
<td></td>
</tr>
<tr>
<td>Salvage (n = 39)</td>
<td>13 (33.3%)</td>
<td>13 (33.3%)</td>
<td>13 (33.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Pearson’s chi-square test: p = 0.81; No salvage group: n = 63 due to lack of information on antibiotic therapy.

Table 2 – Characteristics of groups in which catheter was saved or not, Curitiba, Paraná, Brazil, 2011-2017

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No Salvage group</th>
<th>Salvage group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>mean±SD</td>
<td>median</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>30.4±4.7</td>
<td>31.5±5.7</td>
<td>0.24*</td>
</tr>
<tr>
<td>Prematurity</td>
<td>56 (81.2)</td>
<td>28 (71.8)</td>
<td>0.37*</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1100.0 (465-3655)</td>
<td>975.0 (510-3500)</td>
<td>0.88*</td>
</tr>
<tr>
<td>Length of hospital stay</td>
<td>70 (15-200)</td>
<td>67 (23-169)</td>
<td>0.92*</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>63 (94.0)</td>
<td>35 (89.7)</td>
<td>0.67*</td>
</tr>
<tr>
<td>Previous antibiotic</td>
<td>66 (95.6)</td>
<td>34 (87.2)</td>
<td>0.21*</td>
</tr>
<tr>
<td>Previous invasive procedures</td>
<td>27 (39.1)</td>
<td>18 (46.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>Peripherally inserted central catheter</td>
<td>60 (87.0)</td>
<td>32 (82.0)</td>
<td>0.09*</td>
</tr>
<tr>
<td>Phlebotomy</td>
<td>9 (13.0)</td>
<td>7 (17.9)</td>
<td>0.68*</td>
</tr>
<tr>
<td>Single lumen</td>
<td>61 (92.4)</td>
<td>30 (78.9)</td>
<td>0.09*</td>
</tr>
<tr>
<td>Double lumen</td>
<td>5 (7.6)</td>
<td>8 (21.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>Time of catheterization</td>
<td>16 (6-47)</td>
<td>24 (1-64)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Caliber 1-1.9 (French)</td>
<td>43 (67.2)</td>
<td>24 (64.9)</td>
<td></td>
</tr>
<tr>
<td>Caliber 2 (French)</td>
<td>17 (26.6)</td>
<td>7 (18.9)</td>
<td>0.22*</td>
</tr>
<tr>
<td>Caliber 3-4 (French)</td>
<td>4 (6.2)</td>
<td>6 (16.2)</td>
<td></td>
</tr>
<tr>
<td>Catheter infection time (days)</td>
<td>11 (2-39)</td>
<td>10 (2-39)</td>
<td>0.36*</td>
</tr>
<tr>
<td>Head/jugular/upper limb insertion</td>
<td>55 (83.6)</td>
<td>33 (86.8)</td>
<td></td>
</tr>
<tr>
<td>Lower limb/femoral insertion</td>
<td>11 (16.4)</td>
<td>5 (13.2)</td>
<td></td>
</tr>
<tr>
<td>Positive central blood culture</td>
<td>42 (60.9)</td>
<td>11 (28.2)</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Positive peripheral blood culture</td>
<td>65 (94.2)</td>
<td>37 (94.9)</td>
<td>0.77*</td>
</tr>
</tbody>
</table>

1Student’s T; Pearson chi square/Yates/ Mann-Whitney; 2Pearson chi square. Cases without information: catheter infection time: 3; Insertion location: 5; No salvage: 1; SD = standard deviation

Table 3 – Predictive variables for catheter salvage, Curitiba, Paraná, Brazil, 2011-2017

<table>
<thead>
<tr>
<th>Predictive variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>1.12</td>
<td>0.63-1.97</td>
<td>0.68</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>0.76</td>
<td>0.38-1.54</td>
<td>0.45</td>
</tr>
<tr>
<td>Sex</td>
<td>1.88</td>
<td>0.79-4.46</td>
<td>0.14</td>
</tr>
<tr>
<td>Length of hospital stay</td>
<td>0.94</td>
<td>0.58-1.52</td>
<td>0.82</td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
<td>0.64</td>
<td>0.09-4.17</td>
<td>0.64</td>
</tr>
<tr>
<td>Previous invasive procedures</td>
<td>1.06</td>
<td>0.35-3.23</td>
<td>0.90</td>
</tr>
<tr>
<td>Type (Peripheral Inserted Central Catheter/Phlebotomy)</td>
<td>1.35</td>
<td>0.38-4.82</td>
<td>0.63</td>
</tr>
<tr>
<td>Caliber</td>
<td>0.84</td>
<td>0.34-2.11</td>
<td>0.72</td>
</tr>
<tr>
<td>Insertion location</td>
<td>0.61</td>
<td>0.17-2.20</td>
<td>0.45</td>
</tr>
<tr>
<td>Single lumen catheter</td>
<td>3.20</td>
<td>0.88-11.37</td>
<td>0.07</td>
</tr>
<tr>
<td>Antibiotic therapy scheme</td>
<td>0.85</td>
<td>0.49-1.48</td>
<td>0.58</td>
</tr>
<tr>
<td>Negative central blood culture</td>
<td>3.95</td>
<td>1.69-9.25</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Multivariate Logistic Regression: p < 0.001; OR = Odds Ratio; 95% CI = 95% Confidence Intervals.

DISCUSSION

In the studied sample, very low birth weight NB predominated. Researchers concluded that with each 100 gram decrease in birth weight, the chance of developing CRBSI increased by 1.06 times9. In a study on the risk for bacteremia associated with intravascular devices, half of infections occurred in NB weighing less than 1 kg, thereby predisposing them to an almost five times greater risk of CRBSI compared to those born with more than 2.5 kg10.

There was a higher frequency of catheter infection in NB with GA at birth between 30-33 weeks. For each one week decrease in GA, the chance of CRBSI may double11. The increase in GA has been revealed as a protective factor against CRBSI12.

In the Multivariate Logistic Regression model, the catheter salvage was considered as a dependent variable, while GA, birth weight, sex, length of hospital stay, use of TPN, previous invasive procedures, CVC type and caliber, insertion location, number of lumens, time of catheterization, antibiotic therapy and the positivity of the central blood culture were considered as independent variables.

The negative central blood culture increased the chance of salvage by fourfold (OR = 3.95, 95% CI = 1.69-9.25; p < 0.001), while the single lumen catheter increased by threefold the chance of non-salvage (OR = 3.20, 95% CI = 0.88-11.57) with a borderline significance level (Table 3).
The prevalence of prematurity was quite high, which demonstrates the vulnerability of preterm infants to infections. The data presented are in agreement with recent publications[1,14,28-29].

The RDS and malformations were the most prevalent diagnoses. Premature infants may have clinical complications such as RDS, a condition acting as an adjunct to the risk of infection, as it triggers hypoxemia and acidemia, leading to microbial invasion and worsening of immunodeficiency[30]. Congenital malformations increase the chance of infections, and lead to frequent procedures, prolonged fasting times, use of TPN, antibiotics and invasive devices for many days[31].

Most NB received TPN. The administration of this solution seems to contribute to CRBSI, enhanced by the fact that the integrity of the intestinal mucosa is compromised with the infusion of lipid content[32]. According to authors, the time of TPN was the only risk factor that remained independently associated with confirmed neonatal infection, that is, with each extra day of use, the chance of infection increased by 9%[31].

Many NB received antibiotic therapy prior to the diagnosis of CRBSI. Authors emphasize that a low number of infections is directly related to the restricted use of antimicrobial agents[33]. The empirical use of these drugs changes the newborn’s microbiota, thereby increasing colonization by pathogenic bacteria and predisposing to bloodstream infection in premature infants[30].

Regarding clinical signs of infection, scholars observed that almost all CRBSI events had associated hyperthermia[32]. Others concluded that apnea is a useful clinical sign to guide the investigation of sepsis[1,18]. Researchers have proven that persistent bacteremia was associated with long-term food intolerance[34]. The blood count was infectious and CRP was positive in many events, and studies have related similar laboratory profiles to septic NB[35-36].

The use of PICC predominated over phlebotomy, certainly because the former demonstrates lower risk for infection compared to other vascular catheters and a better cost-benefit ratio when compared to phlebotomy. Furthermore, the PICC has a lower cost than surgically inserted CVCs[7].

Regarding the number of lumens, the insertion of single lumen CVC prevailed. The greater the number of lumens in a device, the greater the chance of CRBSI[10,32]. However, the single lumen catheter increased the chance of catheter salvage by threefold. This result is justified by the low number of double lumen catheters in relation to the amount of single lumen in the studied sample.

The probability of CVC salvage has decreased with CRBSI diagnosed from 15 days of catheterization. This datum is related to the formation of biofilm in a CVC used for more than 48 hours[37]. It is also noteworthy the lower probability that the newborn continues to need a CVC after 15 days of treatment.

The bloodstream infection occurred with approximately ten days of CVC use, which differs from data found in the literature describing the posterior infection of these devices[1,19,21]. This result is directly related to work processes, where multidisciplinary team involvement is an essential condition for successful actions and better quality of care[12].

Few deaths were related to CRBSI. Mortality associated with CNS has not exceeded 1%, and fulminant deaths occurred in patients with other comorbidities when the blood culture result was positive for more than one microorganism[39].

The positive central blood culture was quite high compared to peripheral blood. The interpretation of this difference is based on the difficulty to obtain blood samples from CVCs in neonatology, as they have very small veins and do not show blood flow[28].

Bacterial growth in the central blood culture increased by fourfold the chance of not saving the catheter. More than peripheral, central blood culture is considered the gold standard in the diagnosis of CRBSI, given the higher concentration of bacteria inside the device in relation to blood[41]. A positive central culture is a mandatory indicator of CVC removal.

No therapeutic scheme was more effective than the other for the salvage of catheters. The number of devices saved through antibiotic therapy was low in this study. However, researchers have managed to treat more than 80% of Gram-positive catheters[21,30].

Although there is resistance to oxacillin in many neonatal units, the administration of this drug associated with amikacin was effective in saving the CVC in 13 cases of CRBSI. Brazilian studies prove the efficacy of anti-staphylococcal β lactam to treat CRBSIs[13,38].

The frequency of death in the sample studied was low, as were the cases associated with CRBSI, thus the conclusion that the attempt to salvage infected CVCs did not increase the risk for mortality among NB. Infections by CNS have an insidious evolution and low morbidity and mortality[13].

Limitations of the study

The main limitations of this study were: the retrospective data collection that does not allow researchers to obtain complete and detailed information about participants, since they depend on information from medical records; and the lack of a well-defined protocol in the institution on the treatment of infected CVCs, which allowed different interventions for each professional in the face of a CRBSI diagnosis.

Contributions to the field of Nursing, health or public policies

Considering that NB are patients with very different characteristics from other age groups, the possibility of maintaining vascular access in this vulnerable population becomes relevant for clinical practice and encourages discussions about the safety and effectiveness of the treatment of these devices, since in countless places, removal is mandatory even with the salvage possibility.

CONCLUSIONS

The treatment of CRBSIs resulted in a low prevalence of salvage of CVCs infected by CNS. The probability of CVC salvage was associated with the variables of the venous access device, and was lower when the catheter stayed longer than 15 days and when the result of central blood culture was positive.

SUPPLEMENTARY MATERIAL

This manuscript is the result of a Master’s thesis previously published on the repository of Universidade Federal do Paraná before its submission. Click on the following link to access the full text: https://acervodigital.ufpr.br/bitstream/handle/1184/66267/RF%20-%20DP%20-%20IIZABELA%20LINHA%20SECCO.pdf?sequence=1&isAllowed=y.
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