AN EXPERIMENTAL MODEL OF PNEUMONIA INDUCED BY *METHICILLIN-RESISTENT STAPHYLOCOCCUS AUREUS* IN VENTILATED PIGLETS

Pilar Martínez-Olondris, MD[1]; Oriol Sibila, MD[1]; Carlos Agustí, MD[1]; Montserrat Rigol, PhD[1,2]; Dolors Soy, PhD[3]; Cristina Esquinas, NR[1]; Raquel Piñer, NR[1]; Nestor Luque, MD[1]; Laura Guerrero, Chemist[3]; María Ángels Quera, MD[4], Francesc Marco, MD[4]; Jordi Puig de la Bellacasa, MD[4]; Jose Ramirez, MD[4] and Antoni Torres, MD[1].

AFFILIATIONS
[1]Pneumology Service, Thorax Clinic Institute, Hospital Clinic, IDIBAPS (Institut d'Investigacions Biomèdiques Agustí Pi Sunyer), CIBERES (CIBER de Enfermedades Respiratorias, 06/06/0028), University of Barcelona, Barcelona, Spain; [2]Cardiology Service, Thorax Clinic Institute, Hospital Clinic, Red REDINSCOR RD06/0003/1002, Barcelona, Spain; [3]Pharmacy Service, Hospital Clinic, Barcelona, Spain; [4]CDB, Hospital Clinic, Barcelona, Spain.

CORRESPONDENCE

Antoni Torres Martí

Servei de Pneumologia, Institut Clínic del Tòrax.

Hospital Clínic de Barcelona, Villarroel 170, 08036.

Barcelona, Spain.

Fax: 34 93 2275400, ext. 5549

e-mail: atorres@clinic.ub.es, http://www.idibapsrespiratoryresearch.org.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the first cause of mortality among nosocomial infections [1]. Many aspects regarding the diagnosis, pathophysiology, and therapy of VAP remain unsolved. Animal studies of pneumonia provide a unique opportunity to evaluate some of the incompletely understood mechanisms involved in VAP, without the influence of potential confounders. Marquette et al [2] developed an animal model of pneumonia in mechanically ventilated piglets. This model has proved to be very useful for the proper evaluation of different aspects of VAP [3-9]. Our group, using this animal model, assessed the associated local (lung) and systemic (serum) inflammatory response (IR) in experimental VAP induced by *Pseudomona aeruginosa* after 4 days of mechanical ventilation [10]. Although these studies proved the feasibility of performing animal experiments that are ventilated for a long period of time, they are no exempt of potential shortcomings. Above all, the economic expenses derived from a 4 days long experiment, with the requirement of specific trained personnel to properly manage animals that often are in a critical situation. Also, the potential of contamination with other microorganisms may reasonably increase as the time goes by, further complicating the interpretation of the data. In this sense, shorter experiments would be optimal, particularly if we consider that the precise time course of both the IR of the host to the microbiological inoculation, and the development of histopathological lesions of pneumonia are not known in this animal model. In the present study we aimed to validate a model of VAP in ventilated piglets assessing the time-course of relevant biological markers of inflammation and the histopathological changes of the lung 12 and 24 hours after the inoculation of *methicillin-resistent Staphylococcus aureus* (*MRSA*). We chose this microorganism because is one of the most common causes of nosocomial pneumonia contributing to significant morbidity and mortality [11-12]. Also, the paucity of antibiotics to treat *MRSA* infections, their potential toxicity and variations in lung distribution makes animal models particularly attractive to perform pharmacodinamic and pharmacokinetic studies.

METHODS

Experiments were done in an experimental ICU fully equipped with cardiovascular monitors (Hewlett Packard model 658; Hewlett-Packard, Madrid, Spain), ventilators (Servo 900D, Siemens, Madrid, Spain) and electrical infuser pumps (Juac 591, San Diego, California, USA).

Animal preparation

Twelve healthy domestic-bred Largewhite-Landrace piglets of 3 to 4 month age and mean body weight of 20±2 kg were used in this study. The animals were sedated by an intramuscular injection (2 mg/kg) of azaperone (Stresnil®, Esteve, Barcelona, Spain) and anesthetized with 30 mg/kg intravenous sodium thiopental (Pentotal®, Abbot, Madrid, Spain). They were then orotracheally intubated with a 7.5-mm low-pressure cuff tube (Mallinckrodt 7.5, Mallinckrodt Medical, Athlone, Ireland) and connected to the ventilator. Anaesthesia was maintained with a continuous infusion of midazolam (Midazolam®, Reig-Jofré, Barcelona, Spain) 0.4 $mg \cdot kg^{-1} \cdot h^{-1}$ and phentanyl (Fentanest®, Kern Pharma, Barcelona, Spain) 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ and eventually bolus intravenous of sodium thiopental. A catheter was inserted in the femoral vein 7F (Plastimed, Prodimed, St Leu-la-Forêt, France) for continuous infusion of 5% dextrose solution (0.8 $mL \cdot kg^{-1} \cdot h^{-1}$) and 0.9% saline solution (0.8 $mL \cdot kg^{-1} \cdot h^{-1}$) with an infusion pump. The femoral artery was cannulated with a 3F polyethylene catheter (Plastimed, Prodimed, St Leu-la-Forêt, France) for pressure monitoring and blood sampling. An 8F suprapubic urinary catheter (Rüsch, Kammating, Malaysia) was placed in the bladder through surgical midline minipelvitomy. The piglets were then placed in a prone position and were mechanically ventilated for 12 or 24 hours.

Mechanical ventilation

Animals were mechanically ventilated in a volume-controlled mode. Ventilator parameters consisted of tidal volume (V_T) at a constant inspiratory flow of 10 $mL \cdot kg^{-1}$, a respiratory frequency (fR) of 15 breaths·min^{-1}, an inspiratory time of 33%, with an initial inspiratory fraction (FiO$_2$) of 100% with no positive end-expiratory pressure (PEEP). Later, FiO$_2$ was set according to blood gas analysis in order to obtain arterial oxygen tension (PaO$_2$) >80 and <100 mmHg, and PEEP was increased up to 5 cm H$_2$O when required. Airway pressures,

static lung compliance (calculated by dividing V_T by the difference between the end-inspiratory plateau pressure and the total PEEP [13]) and arterial blood gases (blood gas analyser, AutomaticQC Cartridge, Siemens, Tarrytown, NY, USA) were determined every 6 h. Throughout the protocol, the carbon dioxide arterial tension was maintained at 35–45 mmHg by increasing the fR to the maximum level preceding the appearance of auto-PEEP. Above this limit, hypercapnia was tolerated, as described previously [14].

Bronchial inoculation

Seventy-five mL of 10^6 colony forming units (cfu)·mL^{-1} of pathogenic *MRSA* (strain isolated from patients with Panton Valentine Leukocidin (PVL) status negative and minimal inhibitory concentration (MIC) for vancomycin was 1 mcg·mL^{-1} and 2 mcg·mL^{-1} for linezolid), were inoculated using a fiberbronchoscope and evenly distributed among every lobe of each lung. Inoculations of bacteria were instilled once the animals were haemodinamically stable after sedation and mechanical ventilation.

Sampling and procedures

Mechanical ventilation parameters (V_T, fR, airway pressures and FiO_2), cardiac rate, blood pressure, body temperature, blood gases, serum electrolytes (sodium, potassium) were monitored at 0, 6, 12, 18 and 24 h. Blood biochemistry and blood cells count were obtained at 0, 12 and 24 h.

Bronchoalveolar lavage

Two 15 mL aliquots of sterile saline solution (0.9% sodium chloride) were instilled and re-aspirated through the bronchoscope's channel in the right middle lobe at 0 h (before the inoculation of the *MRSA* suspension) and at sacrifice (12 or 24 h).

Inflammatory parameters

Cytokines in blood and BAL fluid:

Tumour necrosis factor (TNF-α) and interleukin IL-6 and IL-8 levels were measured in serum and BAL supernatant using the ELISA method in specific porcine kits (R&D Systems Inc., Minneapolis, MN, USA).

BAL cytokines were determined at the time of intubation and at the end of the study. Serum cytokines were determined at the time of intubation (baseline) and at 12 and 24 h.

Sacrifice and post mortem studies

Euthanasia was induced at 12 h in 6 piglets and at 24 h in the other 6 piglets. Sacrifice was done under general anaesthesia by intravenous overdose of potassium chloride (40 mEq, rapid iv; B. Braun Medical S.A.).

Collection of lung specimens

After death, the animals remained under mechanical ventilation until surgical samples of lung parenchyma were obtained for bacteriological and histopathological evaluation. The lungs were aseptically exposed through a cervicothoracic midline incision. Thereafter, at least one lung tissue specimen (3 cm^3) was taken from both the more preserved lobe (macroscopically) and the more involved lobe. Specimens were cut into two parts for bacteriological and pathological studies.

Bacteriological studies

Quantitative cultures of BAL fluid, serum and lung tissue were performed at the end of the study. BAL and lung tissue specimens were processed for quantitative bacterial cultures as previously published [15], according to recommended laboratory guidelines [16].

Histopathological assessment

Lung tissue was processed in according with standard methods. Analysis of vessels (thrombosis and endothelial lesions) and pleural (acute or chronic pleuritis) and lung parenchyma was performed. The evaluation of lung parenchyma included severity of pneumonia and presence of other associated lesions (hyaline membranes and alveolar damage). Severity of pneumonia was graded according to previously published criteria [17] in the following grades. 0 = no pneumonia; 1 = purulent mucous plugging; 2 = bronchiolitis; 3 = pneumonia (consolidation coexisting with significant accumulation of polymorphonuclear leukocytes, fibrinous exudates and cellular debris into the

alveolar space); 4 = confluent pneumonia (extension along different secondary lobes); and 5 = abscessed pneumonia (cellular necrosis coexisting with disruption of cellular architecture). Pneumonia was limited to the last three categories. Classification of each specimen was based upon the worst category observed.

Statistical analysis

All data were expressed as mean± standard error (SE). Nonparametric tests for paired data were used; Wilcoxon and Friedman tests were used for comparison of two or more than two time-points, respectively. A p-value < 0.05 was considered statistically significant.

Approval by the Institutional Committee

The study was approved by the Institutional Review Board and Ethics Committee of our Institution; animal care complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and by local government guidelines.

RESULTS

Physiological and laboratory data

Physiological and laboratory variables are shown in table 1. After bronchial inoculation, a rapid and persistent decrease of the static lung compliance ($p<0.05$), mean arterial pressure ($p<0.01$), a rise in body temperature ($p<0.01$) and cardiac rate ($p<0.05$) were observed throughout the study. Moreover, PaO_2/FiO_2 ratio decreased over time, although differences did not reach statistical significance. No differences in biochemical data were observed.

Inflammatory response

Regarding the local IR, a progressive raise in BAL IL-6 concentrations were observed at 12h, which increased further at 24h. Concerning BAL IL-8 and TNF-α concentrations, they also increased significantly compared to baseline but only at 24h (fig. 1). By contrast, in serum, only IL-6 levels showed a significant increase at 24h. The measurement of IL-8 and TNF-α concentrations in serum did not experience any significant change over time (fig. 2).

Microbiology findings

All BAL samples performed at the beginning of the study (before instillation of MRSA) were sterile. By contrast, *MRSA* was present in all the BAL samples collected at the end of the experiment and also in lung tissue cultures of the twelve piglets evaluated. BAL cultures yielded *MRSA* in a concentration .10^3cfu·mL^{-1} in eight piglets and .10^4cfu·mL^{-1} in four piglets. Lung tissue cultures also showed growth of *MRSA* in a concentration .10^3cfu·g^{-1} in all the samples evaluated (fig. 3). Blood cultures were all negative at hour 12 and positive in 5 out of 6 piglets at hour 24. *Pasteurella multocida* and *Staphylococcus coagulase-negative* were also detected in several of the samples, although in low counts.

Histopathological findings

Histopathological evaluation of lung samples evidenced the presence of pneumonia in all the piglets, including those sacrificed at 12h (grades 3–5). However, severe pneumonia, defined as abscessed or confluent pneumonia

(grades 4 and 5), was present in all pigs sacrificed at 24 hours and in only one sacrificed at 12 hours.

DISCUSSION

The main finding of the present study is that histopathological signs of pneumonia and its associated lung inflammatory response (IR) are already evident 12h after the bronchoscopic inoculation of pathogenic *MRSA* in a ventilated piglet. Both, the severity of pulmonary lesions and the intensity of the IR further increase 24h after the inoculation. These findings confirm for the first time the potential utility of this model to study the early effects of antibiotic treatments and coadjuvant therapies against severe *MRSA* pneumonia after 12h of microbial inoculation, decreasing in that way the length and cost of these types of experiments.

Our findings confirm those obtained previously by our own group using *P. aeruginosas* as microbial aetiology of VAP in this animal model (10). Thus, after the inoculation of *MRSA*, animals developed clinical signs of pneumonia (fever, tachycardia, hypotension and gas exchange impairment), and deterioration in lung mechanics (decrease in static compliance over time). Histopatological signs of pneumonia were already evident at 12h although increased at 24h, when all the animals evaluated presented severe pneumonia (defined as abscessed or confluent pneumonia). Also, this study confirms the compartmentalization of the IR and the critical role of IL-6 in the associated IR, as previously described in the same animal model [10]. IL-6 was the only cytokine that increased significantly at 12h in the lung, and the only one that experience significant increased levels in serum. Concentrations of IL-8 and TNF-α in BAL, increased later on, when the severity of pneumonia progressed. To note that IL-6 concentration in serum has been shown to be an independent predictive factor of mortality in different population groups [17-18]. It seems that its determination in serum and BAL, might be a very useful parameter to assess the magnitude of the IR and the potential effects of different anti-inflammatory treatments.

In the present study we have chosen *MRSA* as etiological agent for different reasons. First, Gram-positive pathogens are being reported with increasing frequency as a cause of nosocomial pneumonia mortality [11]. Particularly, *Staphylococcus aureus* is the commonest cause of nosocomial pneumonia and the leading cause of death [19]. This microorganism has developed progressive resistance to β-lactams, and *MRSA* strains emerged in the 1980s [20-21]. Since

then, many institutions throughout the world have reported outbreaks of nosocomial pneumonia caused by *MRSA* [22-23], it is estimated that the risk of death in *MRSA* episodes is 20 times higher than in episodes caused by methicillin-sensitive *S. aureus* [24-25]. Due to the increased prevalence of *MRSA* infection in ventilated patients and the high associated mortality, studies aimed at better knowing both, the pathogenesis of the infection and the effectiveness of antibiotic treatment are of paramount importance.

Different authors have shown that it is possible to develop VAP when piglets are ventilated for as long as 96h [2-10,14]. In the present study we have evidenced, for the first time, that histopathological confirmed pneumonia can be obvious as early as 12h after the inoculation of *MRSA*. Shortening the experiments has obvious potential advantages: First, savings in both financial and human resources are expected since the set up of the experiments requires continuous monitoring of the animals. As a result a large group of ICU professionals (both physicians and nurses) are required. Second, the risk of lung contamination by other micro-organisms and the potential development of extrapulmonary infections increase with time, complicating the proper interpretation of the results obtained. Third, knowing the exact timetable of the apparition of signs of pneumonia after the inoculation of the microorganism may help us to define the precise role of antibiotics in influencing the curse of the infection and the associated IR. This point is particularly relevant when considering *MRSA* pneumonia. The therapeutic options available to treat serious infections due to *MRSA* are limited [26-27]. The emergence of *MRSA* strains with reduced vancomycin susceptibility (*SA-RVS*) further reduces treatment options. Moreover, the pharmacokinetic (PK) profile in ICU patients is frequently too variable to assure the optimal therapeutic outcome by using the standard antibiotic dosage [28-29]. Not only PK but also pharmacodynamics (PD) characteristics influence dosing regimens of antimicrobials. Antibiotic concentrations at the site of the infection differ greatly from those in plasma since drug penetration varies depending on the drug, the tissue involved and also the infection. Thus, in localized infections such as pneumonia, it is extremely important to know what fraction of the free drug will be able to cross the membranes/barriers and reach the site of the infection. The availability of an animal model of *MRSA* pneumonia in which it is possible to perform accurate

PK and PD studies, both in serum and in the lung provides a unique opportunity to gain insight into relevant aspects regarding the response to specific treatments. The increasing recognition of *SA-RVS* will require controlled studies comparing the efficacy of new therapeutic agents, and animal models can be very useful for this specific purpose. In this sense, Luna et al [30] recently published the potential favourable effects of linezolid compared with glycopeptides in *MRSA* pneumonia in piglets. Animals treated with linezolid had a better survival and a trend to better clearance of MRSA, not attributable exclusively to PK/PD effects. Further studies of the efficacy of these new agents against infections caused by *MRSA* or *SA-RVS* are warranted.

The present study has limitations. First, the exogenous administration of highly bacterial inoculums in a previously healthy animal does not necessarily reflect the complexities of pneumonia development in humans. Second, potential species differences in lung immunology between piglets and humans must also be considered. Finally, this is a relative "pure" model of VAP in which animals do not suffer from comorbidities, contrary to what occurs in critically ill patients under mechanical ventilation. However, there are also clear advantages of this animal model, in which the specific role of a particular treatment can be evaluated and PK/PD evaluations can be performed both in serum and in the lung.

In summary, the present study demonstrate that in ventilated piglets, it is feasible to reproduce *MRSA* pneumonia and its associated IR at 12h after the inoculation, with the potential advantages that shortening the experiments may have.

REFERENCES

1- American Thoracic Society; Infectious Diseases Society of America. Guidelines For The Management of Adults With Hospital-acquired, Ventilator-associated and Healthcare-associated Pneumonia. *Am J Respir Crit Care Med* 2005; 171:388-416.

2- Marquette CH, Wermert D, Wallet F, Copin MC, Tonnel AB. Characterization of an animal model of ventilator-acquired pneumonia. *Chest* 1999; 115:200-209.

3- Wermert D, Marquette CH, Copin MC, Wallet F, Fraticelli A, Ramon P, Tonnel AB. Influence of pulmonary bacteriology and histology on the yield of diagnostic procedures in ventilator-acquired pneumonia. *Am J Respir Crit Care Med* 1998; 158:139-147.

4- Goldstein I, Bughalo MT, Marquette CH, Lenaour G, Lu Q, Rouby JJ; Experimental ICU Study Group. Mechanical ventilation-induced air-space enlargement during experimental pneumonia in piglets. *Am J Respir Crit Care Med* 2001; 163:958-964.

5- Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ. Lung deposition and efficiency of nebulized amikacin during Escherichia coli pneumonia in ventilated piglets. *Am J Respir Crit Care Med* 2002; 166:1375-1381.

6- Elman M, Goldstein I, Marquette CH, Wallet F, Lenaour G, Rouby JJ; Experimental ICU Study Group. Influence of lung aeration on pulmonary concentrations of nebulized and intravenous amikacin in ventilated piglets with severe bronchopneumonia. *Anesthesiology* 2002; 97:199-206.

7- Tonnellier M, Ferrari F, Goldstein I, Sartorius A, Marquette CH, Rouby JJ. Intravenous versus nebulized ceftazidime in ventilated piglets with and without experimental bronchopneumonia: comparative effects of helium and nitrogen. *Anesthesiology* 2005; 102:995-1000.

8- Luna CM, Baquero S, Gando S, Patrón JR, Morato JG, Sibila O, Absi R, Famiglietti A, Vay CA, Von Stecher F, Agustí C, Torres A. Experimental severe Pseudomonas aeruginosa pneumonia and antibiotic therapy in piglets receiving mechanical ventilation. *Chest* 2007; 132:523-531.

9- Sibila O, Agustí C, Torres A, Baquero S, Gando S, Patrón JR, Morato JG, Goffredo DH, Bassi N, Luna CM. Experimental *Pseudomonas aeruginosa* pneumonia: evaluation of the associated inflammatory response. *Eur Respir J* 2007; 30:1167-1172.

10- National Nosocomial Infections Surveillance (NNIS) System report, data summary from October 1986 through April 1998. Atlanta: Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1998.

11- Kollef MH, Morrow LE, Niederman MS, Leeper KV, Anzueto A, Benz-Scott L, Rodino FJ. Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia. *Chest* 2006; 129:1210-1218.

12- Rossi A, Polese G, Brandi G, Conti G. Intrinsic positive end-expiratory pressure (PEEPi). *Intensive Care Med* 1995; 21:522-536.

13- Sibila O, Luna CM, Agustí C, Baquero S, Gando S, Patrón JR, Morato JG, Absi R, Bassi N, Torres A. Effects of glucocorticoids in ventilated piglets with severe pneumonia. *Eur Respir J* 2008; 32: 1037–1046.

14- El-Ebiary M, Torres A, González J, de la Bellacasa JP, García C, Jiménez de Anta MT, Ferrer M, Rodriguez-Roisin R. Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator-associated pneumonia. Am Rev Respir Dis 1993; 148:1552-1557.

15- Balows A, Hausler WJJ (Eds): Manual of Clinical Microbiology. 5th Edition. Washington, American Society for Microbiology, 1991.

16- Marquette CH, Copin MC, Wallet F, Neviere R, Saulnier F, Mathieu D, Durocher A, Ramon P, Tonnel AB. Diagnostic tests for pneumonia in ventilated patients: prospective evaluation of diagnostic accuracy using histology as a diagnostic gold standard. *Am J Respir Crit Care Med* 1995; 151: 1878-1888.

17- Bauer TT, Montón C, Torres A, Cabello H, Fillela X, Maldonado A, Nicolás JM, Zavala E. Comparison of systemic cytokine levels in patients with acute respiratory distress syndrome, severe pneumonia, and controls. Thorax 2000; 55: 46–52.

18- Antunes G, Evans SA, Lordan JL, Frew AJ. Systemic cytokine levels in community-acquired pneumonia and their association with disease severity. Eur Respir J 2002; 20: 990–995.

19- Cosgrove SE, Sakoulas G, Prencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis.* 2003; 36: 53-59.

20- Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ. Methicillin-resistant *Staphylococcus aureus* in US hospitals, 1975-1991. *Infect Control Hosp Epidemiol* 1992; 13: 582-586.

21- Maranan MC, Moreira B, Boyle-Vavra S, Daum RS. Antimicrobial resistance in staphylococci: epidemiology, molecular mechanisms, and clinical relevance. *Infect Dis Clin North Am* 1997; 11: 813-849.

22- Oztoprak N, Cevik MA, Akinci E. Risk factors for ICU-acquired methicillin-resistant *Staphylococcus aureus* infections. *Am J Infect Control.* 2006; 34: 1–5.

23- Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. *JAMA* 1995; 274: 639-644.

24- Bodi M, Ardonuy C, Rello J. Impact of Gram-positive resistance on outcome of nosocomial pneumonia. Crit Care Med 2001; 29:82-86.

25- Boucher HW and Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus. Clin Infect Dis.* 2008; 46: S344–S349.

26- Kollef MH. Limitations of vancomycin in the management of resistant Staphylococcal Infections. *Clin Infect Dis* 2007; 45:S191–195.

27- Wunderink RG, Mendelson MH, Somero MS, Fabian TC, May AK, Bhattacharyya H, Leeper KV Jr, Solomkin JS. Early microbiological response to linezolid vs vancomycin in Ventilator-Associated Pneumonia due to methicillin-resistant Staphylococcus aureus. *Chest* 2008; 134:1200–1207.

28- van Dalen R, Vree TB. Pharmacokinetics of antibiotics in critically ill patients. *Intensive Care Med* 1990; 16 (Suppl 3): S235-238.

29- Pea F, Porreca L, Baraldo M, Furlanut M. High vancomycin dosage regimens required by intensive care unit patients cotreated with drugs to improve haemodynamics following cardiac surgical procedures. *J Antimicrob Chemother* 2000; 45(3): 329-335.

30- Luna CM, Bruno DA, García-Morato J, Mann KC, Risso Patrón J, Sagardía J, Absi R, García Bottino M, Marchetti D, Famiglietti A, Baleztena M, Biancolini C. Effect of Linezolid Compared With Glycopeptides in Methicillin-Resistant *Staphylococcus aureus* Severe Pneumonia in Piglets. *Chest* 2009; January, doi: 10.1378/chest.08-2169.

**TABLE 1:** Sequential measurements of physiological and laboratory parameters during the study.

	0 h	6 h	12 h	18 h	24 h	p-value[#]
Temperature ºC	35.4±0.2	36.7±0.3	37.9±0.3	37.4±0.8	37.9±1.1	**<0.001**
Cardiac rate bpm	74±4.6	90±8.1	97±5.3	100±8.5	97±8.2	**0.02**
Arterial pressure mmHg	92±3.8	85±4.2	84±4.1	77±7.5	63±5.6	**<0.001**
CL,s mL·cmH$_2$O^{-1}	21.3±2	19.6±1.6	19±1.9	18.8±3.5	15.8±4.1	**0.02**
Pa,O$_2$/FI,O$_2$ mmHg	467.6±22.9	471.4±31	379.1±38.1	335.8±73.9	376.7±92.4	ns
WBC count $x10^9$ cells·L^{-1}	11.8±1.3		20.6±2.4		12.6±3.7	0.07
Creatinine mg·dL^{-1}	0.8±0.06		0.8±0.07		0.7±0.05	ns

Data are presented as mean±SE, unless otherwise stated. bpm: beats per minute; PaO$_2$: arterial oxygen tension; CL,s: static lung compliance; FiO$_2$: inspiratory fraction; WBC: white blood cell; #: calculated according to the Friedman test. 1 mmHg = 0.133 kPa.

Fig. 1. Sequential determination of BAL a) IL-6, b) IL-8 and c) TNF-α during the study. Data are presented as mean±SE.
*statistical significantly (calculated according to Wilcoxon test).
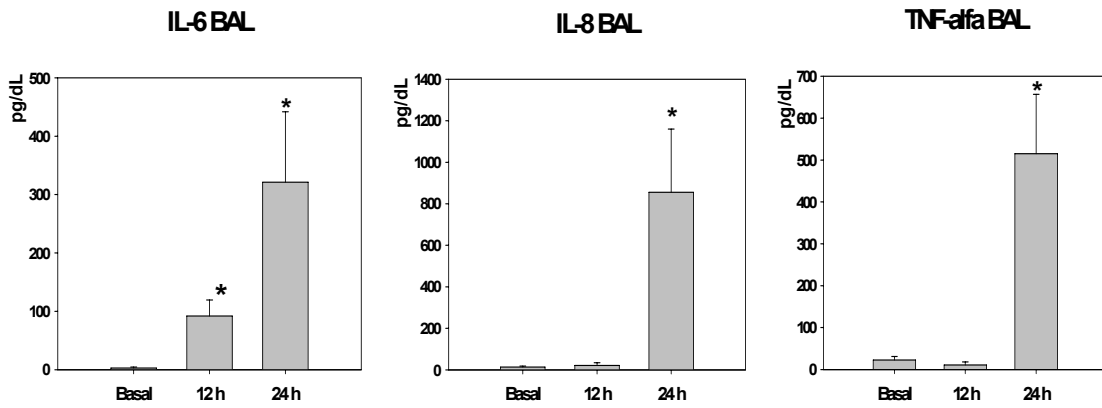
Fig. 2. Sequential determination of serum a) IL-6, b) IL-8 and c) TNF-α during the study. Data are presented as mean±SE.
*statistical significantly (calculated according to Wilcoxon test).

IL-6 serum

IL-8 serum

TNF-alfa serum
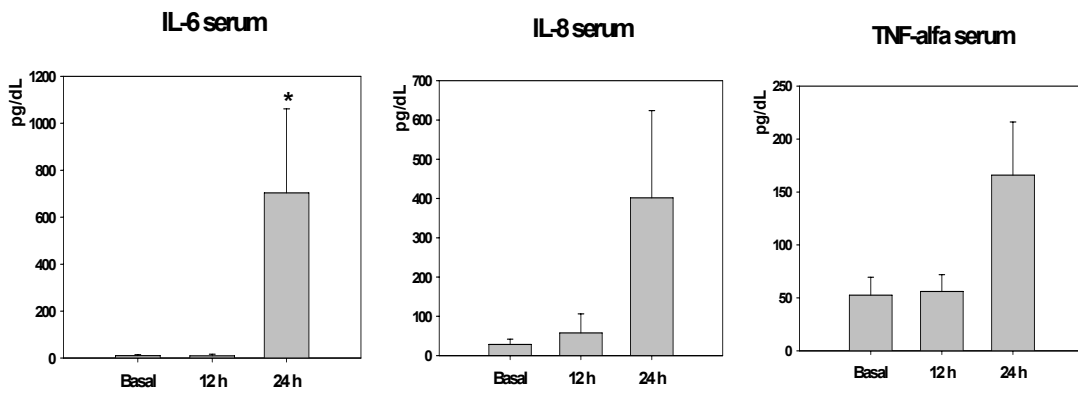
Microbiological findings:

BAL

Lung tissue