Title Page

Incidence of endotracheal tube colonization with the use of PneuX Endotracheal Tubes in cardiac surgical patients

Eshan L Senanayake[1], Ramesh Giri[2], Shameer Goppal[3], Alan Nevill[4], Heyman Luckraz[1]

[1]Department of Cardiothoracic Surgery, [2]Department of Cardiothoracic Anaesthesia, [3]Department of Intensive Care, Heart and Lung Centre, Wolverhampton UK, WV10 0QP & [4]University of Wolverhampton UK, WS

Correspondence to:

Mr. Eshan Senanayake

Heart & Lung Centre

Wolverhampton, UK

WV10 0QP

Tel:+44-190-2694357

Fax:+44-190-2695646

E-mail: eshansenanayake@nhs.net.uk

Manuscript Word Count: 2409

Abstract

Introduction: Ventilator associated pneumonia (VAP) develops in up to 25% of patients after cardiac surgery. Colonisation of the ET tube contributes to VAP. The PneuX endotracheal tube (ETT) has been shown to halve VAP in high-risk patients undergoing cardiac surgery. We report on the secondary analysis of bacterial colonisation in relation to VAP between the PneuX and standard ETTs.

Methods: This was a randomised control trial; Group A (PneuX ET tube, n=120) and Group B (Standard ET tube, n =120). Inclusion criteria; patients > 70 years ± ejection fraction < 50% undergoing cardiac surgery. Incidence of post-operative VAP and analysis of bacterial colonisation within the ETT (n=234) was measured for patients requiring <24hrs, 24-48hrs and > 48hrs of intubation.

Results: Baseline patient demographics were comparable. VAP was lower at 10.8% in group A versus 21% in group B (p=0.03). Incidence of VAP was lower at each time point for group A. There was a lower incidence of ETT colonisation within group A for patients needing > 48hrs intubation. There was no difference in the type of bacterial colonisation (p=0.5) or the mean value of colony forming units; 4.35×10^7 (1.18×10^8) and 2.16×10^8 (1.24×10^9) in A and B respectively (p=0.8).

Conclusion: Colonisation of the ETT does not seem to play an important role in early VAP. There is a tendency for reduced ETT colonisation in the PneuX tube as duration of intubation increases. This may have an impact on reducing the incidence of late-onset VAP.

Abstract word count - 239

Key Words

Ventilator associated pneumonia

Cardiac surgery

PneuX endotracheal tube,

Microbial colonisation

Abbreviations

VAP – ventilator associated pneumonia

ETT – endotracheal tube

LVEF – left ventricular ejection fraction

IQR – inter-quartile range

Introduction

Ventilator associated pneumonia (VAP) is associated with significant morbidity and mortality [1-3], and results in prolonged intensive care and hospital stay leading to serious cost implications. Incidence of VAP has historically been reported at 9-29% in mechanically ventilated patients with the risk of VAP being greatest during the early period of ventilation; early VAP defined as pneumonia that occurs within 4 days of intubation [2, 4, 5]. Risk of death from VAP has decreased with the introduction of preventative care bundles and more recently is reported at 9-13% [6, 7]. Incidence of VAP in the literature is applicable to a heterogeneous group of patients requiring ventilation hence incidence of VAP in cardiac surgical patients is less well reported. Canadian and North American studies estimate VAP to the cost CAN$43 million per year and $11,897 per episode respectively [8].

VAP is inherently linked to endotracheal (ET) intubation and not mechanical ventilation *per se*. The presence of an ETT in the oropharynx results in loss of the natural defence mechanisms of the cough reflex, and the loss of natural anatomic barriers i.e. the glottis and larynx, and further compounded by the placement of foreign material within the respiratory tract [2, 3]. In addition to pre-existing risk factors, pathogenesis of VAP includes microaspiration of bacteria during intubation, development of a biofilm within the ETT which is inaccessible to antibiotic therapy, pooling and trickling of subglottic secretions from above the ETT cuff, and impairment of mucociliary clearance from the lower respiratory tract [2]. Following ET intubation, the limitations of the standard endotracheal tube (ETT) to reduce the above factors, potentiates the risk of developing VAP.

In patients undergoing cardiac surgery the incidence of VAP is estimated to be 3.2 – 8.3% [9]. Furthermore other risk factors for developing VAP following cardiac surgery included age >

70 years, impaired ventricular function, and those intubated for >48hrs [10]. The PneuX ETT has a number of features that can reduce VAP. The PneuX ETT features a low-volume low-pressure silicone cuff. This tube is unique in that the cuff inflates uniformly circumferentially without any micro-folds within the cuff thereby providing a watertight seal between the cuff and the tracheal mucosa. In a recently published in-vitro study the PneuX tube was the only ETT compared to seven other ETT systems to prevent microaspiration of subglottic contents [11]. The PneuX system also has a continuous tracheal seal monitor that measures and maintains the endotracheal tube cuff pressure at 20 -30mmHg. Like a number of other ETT systems with subglottic suction the PneuX system has three subglottic suction ports to allow suglottic suction and irrigation of secretions that pool above the cuff. The PneuX system also has a non-stick parylene coating on the inner lumen, that may help reduce biofilm formation. However the impact of this coating on the PneuX system on biofilm formation and subsequent ETT colonisation has not previously been investigated.

The NASCENT trial showed a reduction of microbiologically confirmed VAP from 7.5% to 4.8% with the use of Silver hydrogel coated ETT [12]. Silver hydrogel helps reduce biofilm formation on the inner lumen of the ETT [2, 8]. Similarly non-stick coatings can help reduce biofilm formation [13] and reduction of biofilm formation is associated with VAP reduction [3]. ETT biofilm formation is abundant at 96hrs from intubation and can act as a reservoir of pathogens for recurrent infections. Retrograde ETT colonisation has been hypothesised as one mode of tube colonisation. Pathogens that colonise the ETT have shown to be consistent with the same pathogen being isolated from endotracheal aspirates, 70% of the time [14].

Our group have shown in a RCT of high-risk patients undergoing cardiac surgery, that there is a significant reduction in VAP from 21% to 10.8% with the PneuX ETT compared to standard ETT [15]. Here we report on the sub-analysis of the extent of microbial colonisation within the PneuX ETT versus the standard ETT, in high-risk patients undergoing cardiac surgery and assesses the impact of this on VAP. Furthermore we attempt to elucidate the association with ETT bacterial colonization and VAP.

Methods

This study was designed as randomised controlled trial, randomising 1:1 to either the PneuX ETT (Group A) or the standard ETT (Group B). The study was authorised by the Hospital R&D department and was approved by the Ethical committee (10/H1208/42). It was registered with the ISRCT (45757289) and UKCRN portfolio (9831). The study followed the Good Clinical Practice Guidelines and Declaration of Helsinki 1964 (amended Edinburgh 2000).

Inclusion criteria included patients over the age of 70 years ± impaired left ventricular function (LVEF <50%) undergoing elective and urgent cardiac surgery. Exclusion criteria included those who had a pre-op chest infection prior to surgery or underwent emergency surgery. Patients were randomized using a using a computer generated randomization software program developed by the Trans European Network for Clinical Trial Services, (TENALEA, Amsterdam, Netherlands). Standard study protocols for anaesthesia including peri-operative antibiotic cover, surgery and postoperative care have been reported previously [15] and were standardized for each group. In patients randomized to the PneuX ETT, the study protocol followed the manufactures guidelines for the Venner-PneuX VAP prevention system. This included connecting the Pneu-X ETT to the Venner tracheal seal

monitor, which maintained a cuff pressure of 20-30mmHg. Furthermore the sub-glottic ports were irrigated 6 hourly with 10ml of distilled sterile water as per the manufactures guidelines until the aspirates were clear. Patients that require re-intubation received the same ETT as initially randomised. Similarly if a patient subsequently required a tracheostomy the same randomisation would be adhered to i.e. standard tracheostomy tube or PneuX tracheostomy tube.

Patients were observed and assessed for clinical evidence of VAP for the duration of their period of intubation and for 48 h after extubation. A diagnosis of VAP was confirmed via the HELICS definition [16]. When the patient was extubated, the ETT was placed on an aseptic area and 1cm of the distal end of the endotracheal tube was cut to exclude any contamination during extubation i.e. on contract with oropharynx, teeth and lips. A standard microbial swab was then taken from within the inner surface of the remaining distal end of the ETT under aseptic conditions and immediately sent for microscopy, sensitivity and culture. An independent microbiology reference laboratory in Birmingham UK, assessed the type of bacteria grown and the number of total bacterial colony forming units grown.

Statistical analysis

The primary aim of the study was the incidence of VAP and to assess the primary end point of the incidence of VAP, using a power of 90% and alpha of 0.01 proved that at least 107 patients were required per group [15]. Here we report on the secondary outcome measure: analysis of the microbial colonisation (number of colony forming units) between the PneuX and standard ETT and the relation to the incidence of VAP with each ETT.

Statistical analysis was performed on SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Categorical variables are expressed as a percentage and differences between groups assessed using the Chi-squared test of independence. Continuous variables are expressed as mean ± standard deviation or median and inter-quartile range (IQR). Group comparison was carried out using student's t-test for normally distributed data or non-parametric tests for skewed data. Significance was defined as p<0.05.

Results

Microbial analysis was possible on 234 patients (97.5%, 234/240). There was no significant difference in demographics between the groups (Table 1) with a EuroSCORE of 6.39 (SD 2.2) and 6.48 (SD 2.6) in groups A and B respectively (p=0.9). The incidence of impaired LV was 37% and 39% in groups A and B respectively. The primary end point and analysis of this end point has been previously reported [15]. There was a significantly lower total incidence of VAP within group A (PneuX ETT) of 10.8% compared to 21% in group B (p=0.03). Furthermore the PneuX ETT was associated with a significant reduction of VAP (Odds ratio 0.45, P = 0.03). Other clinical post-operative outcomes were comparable between the groups with a median intubation time of 14.7 (IQR 7.3 – 2927.2) hours and 13 (2.5 – 528.7) hours in groups A and B respectively and no difference in ICU length of stay and overall mortality (p=0.2).

Assessment of microbial analysis showed that of the 92% (84/91) patients had bacterial colonisation with an intubation time < 24hrs, 9% (8/91) developed VAP in group A. In comparison in the same time frame (<24hrs intubation) 78% (75/94) developed bacterial colonisation with the standard ETT and 16% (14/96) of patients developed VAP (Figure 1).

The incidence of VAP was higher in patients that were intubated for longer; 24 – 48hrs of intubation had a VAP incidence of 0% (0/18) and 33% (5/15) in group A and B respectively and at > 48hrs of intubation, the incidence of VAP was even greater at 48% (3/7) and 71% (5/7) in groups A and B respectively. In group A (PneuX ETT) the percentage of patients with microbial colonisation was less in patients that were intubated for longer; reduction from 94% (84/91) at 24-48hrs intubation to 57% (4/7) at >48hrs intubation. In contrast, in group B the percentage of patients with microbial colonisation was higher in those intubated for longer; increment from 73% (75/96) at 24-48hrs intubation to 86% (6/7) at >48hrs intubation (Figure2). The incidence of VAP was consistently lower at each time point for group A, compared to group B. With intubation times of <24hrs and 24-48hrs, despite the high percentage of colonisation the incidence of VAP was low in group A. The percentage of colonisation for each tube was comparable between those that require <24hrs and 24-48hrs of intubation.

There was no significant difference between the groups in the mean number of colony forming units with 4.35×10^7 (1.18×10^8) and 2.16×10^8 (1.24×10^9) in groups A and B respectively (p=0.8) (Figure 2). Similarly there was no difference in the in the types of bacterial colonisation between the two ETTs (Table 2).

Discussion

As previously reported the PneuX ETT is associated with a lower incidence of ventilator associated pneumonia in high risk patients undergoing cardiac surgery [15]. Furthermore in this study we have shown that, despite no significant difference in mean number of colony forming units between the standard and PneuX ETT, there is a trend towards reduced

microbial colonisation within the PneuX ETT in patients that require longer intubation. Furthermore there is a lower incidence of VAP with the PneuX ETT irrespective of the duration of intubation. Moreover, with short intubation times (<48hrs) colonisation of the ETT does not share a direct correlation with the incidence of VAP post cardiac surgery. The majority of patients undergoing cardiac surgery envisage intubation times of <48hrs, however in higher risk patients longer mechanical ventilation may be required. In these patients, devices that can help reduce VAP would be prudent.

Pathogen colonisation of the ETT and biofilm formation has been described to occur through retrograde colonisation of the ETT and ventilator tubing that can even contaminate the condensate [3, 8] and can occur within hours to a few days of intubation [14, 17-19]. A biofilm can be defined as a complex aggregation of microorganisms growing on the surface of the ETT [3], hence protected from any immune response or systemic antibiotics. Therefore biofilm formation initiates a vicious cycle of events by repeated inoculation of the lower respiratory tract with each ventilator cycle perpetuating existing infection. Furthermore these organisms can be dislodged from the tubing during endotracheal suctioning. Following the NASCENT study by Kollef and colleagues there is strong evidence (36% risk reduction) in favour of reducing incidence of VAP with a silver-coated ETT. The PneuX ETT although not silver-coated, has a non-stick inner layer that inhibits the adhesion of biological materials. In this study we have demonstrated that over time, the incidence of VAP is lower with the PneuX ETT and concurrently the percentage of microbial colonisation reduced from 94% at 24-48hrs to 57% at > 48hrs. This trend is opposite to that observed with the standard ETT. The trend in colonisation over time, within a standard ETT is consistent to previous reports, where colonisation has been described to be abundant by 96hrs [14, 18, 19]

The microorganisms involved in VAP are dependent on duration of intubation; typically early VAP commonly caused by *Streptococcus pneumonia,* Haemophilus influenza *Staphylococcus aures* and *Escherichia coli.* Multi-drug resistant organisms such as Acinetobacter, Pseudomonas aeroginosa, commonly cause late VAP [18, 20, 21]. This study showed no difference in the type of organism being isolated between the standard and PneuX ETT. Furthermore the number of colony forming units between the groups showed no statistically significant difference *albeit* with a large standard deviation within the standard ETT group. This leads to the hypothesis that the incidence of VAP within each group is associated with the percentage of colonisation, irrespective of the number and type of organism being isolated, especially as the duration of intubation increases.

Biofilm formation has been reported as one of 2 main mechanism of VAP [1]. Assuming that ETT colonisation and biofilm formation occurs retrogradely, it is prudent to note that colonization of the PneuX ETT is not exclusively due to the non-stick inner layer, but secondary to a number of features described earlier that reduces the incidence of VAP through reduction in microaspiration (the other main cause of VAP). Therefore reduction in VAP should target all the likely causes of VAP; biofilm formation is known to potentiate VAP and not be the precipitating cause of VAP [21]. This is further substantiated by evidence from the NASCET trial showing that apart from the incidence of VAP, there was no difference in duration of mechanical ventilation or length of intensive care or hospital stay [12] with a silver coated ETT alone.

An ETT that has a lower colonisation potential in those that require longer intubation has greater benefit in those patients requiring mechanical ventilation for > 48hrs. These patients may have other risk factors such as chronic pulmonary disease, sepsis, acute

respiratory distress, neurologic disorders, blood transfusions [4]. Electing to use a VAP lowering ETT in these high-risk patients alone could provide an overall cost advantage to offset the higher initial cost of the ETT. Shorr and colleagues have demonstrated the cost-effectiveness of using silver-coated ETT, with a cost saving of $12,840 per case of VAP prevented [22]. Similarly a study to evaluate the cost-effectiveness in using the PneuX ETT with features that reduce both microaspiration and biofilm formation is required. Within the cohort of patients undergoing cardiac surgery, most would expect to be extubated by 48hrs; therefore such a cost-effectiveness study will help clarify whether the PneuX ETT has a overall cost benefit for those considered as high risk and/or those that require intubation for >48hrs or even for all comers.

This study was undertaken on a selected cohort of patients that included those at high-risk and undergoing cardiac surgery. This does not reflect the general population who may require intubation for longer and may have other risk factors outlined earlier for developing VAP. However, even in this select group of patients, there is a trend towards decreased colonisation and incidence of VAP compared to standard ETT when patients required a longer intubation and this can be extrapolated to the general population who may benefit further from using the PneuX ETT.

In conclusion colonisation of the ETT does not seem to play an important role in early VAP and this may be due to the short intubation time. Given the features of the PneuX ETT, which help reduce VAP, there is a tendency for reduced ETT colonisation in the PneuX tube in patients that require a longer intubation. This reduced late colonisation within the PneuX ETT may have an impact on reducing the incidence of late-onset VAP in patients that require intubation for > 48hrs.

References

1. Fernandez JF, Levine SM, Restrepo MI. Technologic advances in endotracheal tubes for prevention of ventilator-associated pneumonia. *Chest.* Jul 2012;142(1):231-238.

2. Kalanuria AA, Zai W, Mirski M. Ventilator-associated pneumonia in the ICU. *Crit Care.* 2014;18(2):208.

3. Young PJ, Doyle JA. Preventing ventilator-associated pneumonia - The role of the Endotracheal Tube. *Current Respiratory Medicine Reviews.* 2012(8):170-183.

4. American Thoracic Society IdsoA. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *American journal of respiratory and critical care medicine.* Feb 15 2005;171(4):388-416.

5. Hunter JD. Ventilator associated pneumonia. *BMJ.* 2012;344:e3325.

6. Melsen WG, Rovers MM, Groenwold RH, et al. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *The Lancet. Infectious diseases.* Aug 2013;13(8):665-671.

7. Melsen WG, Rovers MM, Koeman M, Bonten MJ. Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies. *Critical care medicine.* Dec 2011;39(12):2736-2742.

8. Zolfaghari PS, Wyncoll DL. The tracheal tube: gateway to ventilator-associated pneumonia. *Crit Care.* 2011;15(5):310.

9. Bouza E, Perez A, Munoz P, et al. Ventilator-associated pneumonia after heart surgery: a prospective analysis and the value of surveillance. *Critical care medicine.* Jul 2003;31(7):1964-1970.

10. Hortal J, Munoz P, Cuerpo G, Litvan H, Rosseel PM, Bouza E. Ventilator-associated pneumonia in patients undergoing major heart surgery: an incidence study in Europe. *Crit Care.* 2009;13(3):R80.

11. Mariyaselvam M, Marsh L, Wise M, Williams D. An in vitro microbiological study comparing eightendotracheal tubes and their ability to preventmicroaspiration. *Intensive Care Medicine Experimental.* 2015;3(Suppl 1):A382.

12. Kollef MH, Afessa B, Anzueto A, et al. Silver-coated endotracheal tubes and incidence of ventilator-associated pneumonia: the NASCENT randomized trial. *Jama.* Aug 20 2008;300(7):805-813.

13. Fletcher AJ, Ruffell AJ, Young PJ. The LoTrach system: its role in the prevention of ventilator-associated pneumonia. *Nursing in critical care.* Sep-Oct 2008;13(5):260-268.

14. Adair CG, Gorman SP, Feron BM, et al. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. *Intensive care medicine.* Oct 1999;25(10):1072-1076.

15. Gopal S, Luckraz H, Giri R, et al. Significant reduction in ventilator-associated pneumonia with the Venner-PneuX System in high-risk patients undergoing cardiac surgery: the Low Ventilator-Associated-Pneumonia study. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery.* Mar 2015;47(3):e92-96.

16. Hospitals in Europe Link for Infection Control through Surveillance. Accessed 16th December, 2009.

17.   Deem S, Treggiari MM. New endotracheal tubes designed to prevent ventilator-associated pneumonia: do they make a difference? *Respiratory care.* Aug 2010;55(8):1046-1055.

18.   Feldman C, Kassel M, Cantrell J, et al. The presence and sequence of endotracheal tube colonization in patients undergoing mechanical ventilation. *The European respiratory journal.* Mar 1999;13(3):546-551.

19.   Gorman S, Adair C, O'Neill F, Goldsmith C, Webb H. Influence of selective decontamination of the digestive tract on microbial biofilm formation on endotracheal tubes from artificially ventilated patients. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology.* Jan 1993;12(1):9-17.

20.   Abdollahi A, Shoar S, Shoar N. Microorganisms' colonization and their antibiotic resistance pattern in oro - tracheal tube. *Iranian journal of microbiology.* Jun 2013;5(2):102-107.

21.   Gil-Perotin S, Ramirez P, Marti V, et al. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. *Crit Care.* 2012;16(3):R93.

22.   Shorr AF, Zilberberg MD, Kollef M. Cost-effectiveness analysis of a silver-coated endotracheal tube to reduce the incidence of ventilator-associated pneumonia. *Infection control and hospital epidemiology.* Aug 2009;30(8):759-763.

Table 1 – Pre-op patient demographics

	Standard ET tube (n=120)	Venner-PneuX tube (n=120)	p-Value
Age* (years)	72 (7)	72 (8)	0.5
Male (n, %)	91, 77%	83, 69%	0.2
Elective (n, %)	78, 65%	81, 67%	0.8
Lung Disease (n, %)	22, 18%	20, 17%	0.7
PVD (n, %)	31, 26%	20, 17%	0.08
Diabetes (n, %)	31, 26%	44, 37%	0.07
Recent MI (n, %)	28, 25%	18, 21%	0.2
Isolated CABG (n, %)	73, 61%	62, 52%	0.3
Impaired LV (n, %)	46, 39%	44, 37%	0.09
Euroscore*	6.4 (2.6)	6.4 (2.2)	0.9

*denotes data expressed as mean (standard deviation)

Table 2 – Types of microbial colonisation between the groups

	standard ETT	PneuX ETT	p = 0.5
GPC	42%	35%	
GNC	14%	17%	
GNR	39%	44%	

GPC: gram positive cocci

GNC: gram negative cocci

GNR: gram negative rods

Figure Legend

Figure 1 – Incidence of microbial colonisation and VAP between standard and PneuX ETT

Figure 2 – Total microbial load between Standard ETT and PneuX (LoVAP) ETT