Analysis of Clinical Outcome of Synthetic Tracheal Transplantation Compared to Results Published in 6 Articles by Macchiarini et al.

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Contents

Introduction

The analysis is initiated with a table describing the patients who have received a synthetic trachea transplant at Karolinska University Hospital. This is followed by a chronological review of 6 articles published by Macchiarini et al. The first article is the primary reference of synthetic trachea transplantation in humans and is the cornerstone on which the other articles stand.

Analysis of the individual articles reviews clinical data and the conclusions that the writers have reached and compares these to the information that is registered in the patients' medical records. Only information in the medical records from Karolinska University Hospital or information provided directly by Prof Macchiarini's research group has been utilized. Individual articles are divided into their own section, which starts with a list of points of greatest significance, which we have termed "Major Inconsistencies". Details and analysis which support these points follows in the sections titled "Inconsistent and Omitted Clinical Data". This analysis explores different issues in the order that they appear in each individual article in order to maintain a sense of continuity. The most important diagnostic procedures or interventions have been written in bold font. A "General Comments" section then follows which reiterates the findings and implications of each point. Again issues of greater significance are marked in bold font.

Attached at the end of the analysis under the section titled "Appendixes" are copies of published articles and medical records used as references in the findings and conclusions of the analysis. Bronchoscopic films have been digitalized and collected on a USB mass storage device which accompanies the analysis.

Permission for full access of the medical records has been attained from the families of Case 1 and 3 and have been filed in the patients' medical records. We have been unable to locate the family of Case 2 who resided in the US, so unfortunately no written permission has been attained.

Patient Characteristics, Complications and Outcome

PATIENTS	CASE 1	CASE 2	CASE 3
Residency	Iceland	USA	Turkey
Gender	Male	Male	Female
Age	36	30	22
Etiology of Tracheal disorder	Malignancy	Malignancy	Iatrogenic Injury
PRIMARY PROCEDURE	Synthetic Trachea	Synthetic Trachea	Pulmectomy + Synthetic Trachea
Date of Procedure	Jun 9, 2011	Nov 17, 2011	Aug 7, 2012
Scaffold material	POSS/PCU	PET/PU; 70/30	PET/PU; 70/30
POST-TRANSPLANT COMPLICATIONS			
Transplant associated complications/outcome			
Transplant associated granulations	Yes (significant)	Yes (scantly)	Yes (significant)
Anastomotic (transplant) dehiscence	Yes (all anastomosis, disconnected transplant)	? (see under tracheo-mediastinal fistula)	Yes (distal anastomosis)
Tracheo-esophageal fistula	Yes	No	Yes
Tracheo-mediastinal fistula	Yes	? (suspected in CT report Jan 10, 2012)	Yes (massive air leakage out of thoracotomy)
Transplant material fatigue/collapse	No	No	Yes
Near fatal airway (stent) occlusion	Yes (terminally)	No	Yes (multiple occasions)
Normal airway epithelium in transplant	No	No	No
Chronic infection	Yes (mediastinitis)	-	Yes (thoracic rest cavity)
General complications			
Respiratory failure	Yes	Yes	Yes
Pneumonia (P), Abscess (A)	Yes (Chronic P, A)	Yes (P)	Yes (P)
Sepsis	Yes	Yes	Yes
Hemoptysis	Yes	Yes	No
Thrombo-embolic events	Yes	Yes	Yes
Acute (A) Chronic (C) Renal Failure	Yes (A) (terminally)	No	Yes (A, C)
Splenic Infarction	No	No	Yes
Multiple Organ Dysfunction Syndrome	Yes (terminally)	No	Yes
POST-TRANSPLANT INTERVENTIONS/THERAPY			
Airway stenting (multiple interventions)	Yes	No	Yes
Bronchoscopy dependency	Yes (intermittent)	No	Yes (every 4th hour, 24-7)
Chronically Tracheotomized	No	No	Yes (2 years)
Esophageal stenting	Yes	No	Yes
Esophagectomy	Yes (stapled transection)	No	Yes
Thoraco-plasty incl. Pedicled Lat. Dorsi Flap	No	No	Yes
Chronic Thoracic Drainage	No	No	Yes
Nutrition through PEG/Gastrostomy	Yes (partly)	No	Yes (2years)
Laparotomy	Yes	No	Yes (3 times)
Chronic Antibiotic/Antifungal therapy	Yes (intermittent)	No	Yes
Extracorporeal Membrane Oxygenation	No	No	Yes (3 times, in tot. 72 d.)
Hemodialysis	No	No	Yes (7 weeks)
Prolonged ventilator dependency	Yes	No	Yes (305 days)
RE-TRANSPLANTATION	No	No	Synthetic Trachea
Date for re-transplantation	-	-	Jul 9, 2013
Scaffold material	-	-	PET
Post-re-transplant stenting (multiple occasions)	-	-	Yes (custom made stents)
FINAL OUTCOME			
Dead/Alive, Date	Died Jan 30, 2014 (after 8 months of hospitalization)	Died Mar 5, 2012	Alive (hospitalized in the ICU after more than 2 years)
Cause of Death	Refractory respiratory insufficiency, transplant disconnection	Airway bleeding? Tracheo-arterial fistula?	-
Total numbers of transplant associated	32	12	139

surgical interventions in the Karolinska records			(until August 2014)

Articles Reviewed

1. **Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study.**
   Jungebluth P, Alici E, Baiguera S, Le Blanc K, Blomberg P, Bozóky B, Crowley C, Einarsson O, Grinnemo KH, Gudbjartsson T, Le Guyader S, Henriksson G, Hermanson O, Juto JE, Leidner B, Lilja T, Liska J, Luedde T, Lundin V, Moll G, Nilsson B, Roderburg C, Strömblad S, Sutlu T, Teixeira AI, Watz E, Seifalian A, Macchiarini P. *Lancet*. 2011 Dec 10; 378(9808):1997-2004.
   Submitted Oct 11, 2011, published online Nov 24, 2011.
   (Appendix 4).

2. **Engineered whole organs and complex tissues.**
   Badylak SF, Weiss DJ, Caplan A, Macchiarini P. Lancet. 2012 Mar 10; 379(9819):943-52. Review

   Submitted Aug 12, 2011, published online Mar 10, 2012.

   (Appendix 10).

3. **Verification of cell viability in bioengineered tissues and organs before clinical transplantation.**
   Jungebluth P, Haag JC, Lim ML, Lemon G, Sjöqvist S, Gustafsson Y, Ajalloueian F, Gilevich I, Simonson OE, Grinnemo KH, Corbascio M, Baiguera S, Del Gaudio C, Strömblad S, Macchiarini P. *Biomaterials*. 2013 May; 34(16):4057 2013.02.057. Epub 2013 Mar 6.

   Submitted Feb 5, 2013, published online Mar 6, 2013.

   (Appendix 9).

4. **Are synthetic scaffolds suitable for the development of clinical tissue-engineered tubular organs?**

   Del Gaudio C, Baiguera S, Ajalloueian F, Bianco A, Macchiarini P. *J Biomed Mater Res A.* 2014 Jul;102(7):2427-47. Epub 2013 Aug 2.

   Submitted Mar 18, 2013, published online Aug 2, 2013.

   (Appendix 11).

5. **Airway transplantation.**

   Jungebluth P, Macchiarini P. *Thorac Surg Clin.* 2014 Feb; 24(1):97-106. Review.

   Submitted Aug, 2013, published online Feb 24, 2014.

   (Appendix 12).

6. **Biomechanical and biocompatibility characteristics of electrospun polymeric tracheal scaffolds.**

   Ajalloueian F, Lim ML, Lemon G, Haag JC, Gustafsson Y, Sjöqvist S, Beltrán-Rodríguez A, Del Gaudio C, Baiguera S, Bianco A, Jungebluth P, Macchiarini P. *Biomaterials.* 2014 Jul; 35(20):5307-15. Epub 2014 Apr 3.

   Submitted Jan 13, 2014, published online Apr 3, 2014.

   (Appendix 13).

Articles

Article 1

Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study.

Jungebluth P, Alici E, Baiguera S, Le Blanc K, Blomberg P, Bozóky B, Crowley C, Einarsson O, Grinnemo KH, Gudbjartsson T, Le Guyader S, Henriksson G, Hermanson O, Juto JE, Leidner B, Lilja T, Liska J, Luedde T, Lundin V, Moll G, Nilsson B, Roderburg C, Strömblad S, Sutlu T, Teixeira AI, Watz E, Seifalian A, Macchiarini P. *Lancet.* 2011 Dec 10; 378(9808):1997-2004.
(Appendix 4).

Submitted Oct 11, 2011, published online Nov 24, 2011.

Major Inconsistencies:

1. Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board.

2. Unapproved informed consent form signed 17 days after the transplantation.

3. Serial fabrication and omission of biopsy findings.

4. Serial fabrication and omission of bronchoscopic findings.

5. Fabrication and omission of clinical status.

Inconsistent and Omitted Clinical Information:

1. The *Abstract in the Findings section* included the following statements: ***"…There were no major complications, and the patient[1] was asymptomatic and tumour free 5[2] months after transplantation. The bioartificial nanocomposite has patent anastomoses, lined with a vascularised neomucosa, and was partly covered by nearly healthy epithelium.*** *Postoperatively, we detected a mobilisation of peripheral cells displaying increased mesenchymal stromal cell phenotype, and upregulation of epoetin receptors, antiapoptotic genes, and miR-34 and miR-449 biomarkers. These findings, together with increased levels of regenerative-associated plasma factors, strongly suggest stem-cell homing and cell-mediated wound repair, extracellular matrix remodeling, and neovascularisation of the graft."*
   [1]Refers to Case 1 who was transplanted on Jun 9, 2011. The patient died at Karolinska on Jan 30, 2014.
   [2]Approx. beginning of Nov 2011.

   - Concerning the stated ***"asymptomatic"*** patient:

     At readmission to Karolinska University Hospital on Nov 21, 2011, 5½ months after transplantation and 3 days before publication of the article on Nov 24, 2011, the medical records describe the clinical admission state of the patient as follows: ***"looks a bit worn out", "productive sputa", "states that he has lost 7kg after the operation", "no respiratory sounds on the right chest wall, relatively clear respiratory sound on the left side."***
     (Nov 21, 2011, Clinical notes from admission Appendix 14).

     Comments:
     The statement in the article that ***"the patient was asymptomatic…5 month[1] after transplantation"*** is contradicted by the medical records, which described a severely symptomatic patient at submission.
     [2]Approx. beginning of Nov 2011.

   - Concerning the statement in the article: ***"…5 months after transplantation. The bioartificial nanocomposite has patent anastomoses, lined with a vascularised neomucosa, and was partly covered by nearly healthy epithelium"*** the bronchoscopy findings registered in the Karolinska medical records reveals the following:

a. **Bronchoscopy on Nov 21, 2011 (on the date of re-admission)** 5½ months after transplantation and 3 days *before* publication of the article on Nov 24, 2011, describes:
4th line: *"there are distal granulations on the distal graft endings on both the right and left side. Starting by inspecting that there is a passage down in the left main bronchus which is stenosed but not more than to maybe 20%."*
8th line: *"I first go down and recess the granulations on the right side in the main bronchus. It is possible to reduce them. They bleed fairly easily but it is possible to reduce the masses, it is not possible to clearly identify the branching of the upper lobe."*
14th line: ***"Decide to implant a stent…"*** (Right main bronchus).
19th line: *"One can suspect* ***a small opening at 1 o'clock position,*** *in other words inside medially in front exactly at the edge of the graft.* ***Implant even here a stent…"*** (Left main bronchus).
(Nov 21, 2011, Bronchoscopy report, Appendix 15).
(Nov 21, 2011, Bronchoscopy Film 1 on the USB device).

b. **Bronchoscopy was repeated the next day on Nov 22, 2011, with the following findings:**
4th line: *"…the right bronchus is* ***filled with pus****. It looks better on the left side. Sucking clean. It fills up all the time from down below on the right side."*
12th line: ***"…serous fluid is entering through the anastomosis between graft and bronchus at 6-7 o´clock position, right side."***
15th line: *"…****inserting a new stent****…"* (Right main bronchus).
23rd line: *"****See fistulation*** *which lies medially and at the edge of the graft, on this side at 2-3 o´clock position."* (Left main bronchus).
24th line: *"Finish by pulling the stent up again so that I achieve a nice cover in applied and treated surfaces."* (Left sided stent).
(Nov 22, 2011, Bronchoscopy report, Appendix 17).
(Nov 22, 2011, Bronchoscopy Film 2 on the USB device).

Comments:
The bronchoscopy findings from Nov 21-22, 2011 with significant granulations in the anastomotic regions, verified fistulation, need for stent implantation and stent repositioning totally contradict the statements in the article of *"patent anastomoses…"*
Prof Macchiarini was present at the ENT-Department according to the medical records of Nov 21, 2011, and must thereby have been fully aware of the clinical status and the bronchoscopic findings and the subsequent need for intervention.
(Nov 21, 2011, Medical record note, Appendix 16).

- Concerning the statement in the article that the transplant was **"*lined with a vascularised neomucosa, and was partly covered by nearly healthy epithelium*"**:
There are no biopsies recorded in the Karolinska University medical records that confirm biopsy analyses at the stated "*5 months after transplantation".* The temporally nearest biopsies registered in the Karolinska medical records were taken on Dec 20, 2011 (which is 6½ months ***after transplantation***, 2½ months ***after submission*** of the article on Oct 11, 2011 and 4 weeks ***after publishing*** of the article on Nov 24, 2011) with the following findings:

c. **Biopsy report from Dec 20, 2011 (6½ months after transplantation), describes:**
The nature of the sample: "mucus membrane/granulation from the trachea"

Diagnostic Query*: "granulation? ca?"*
Description of the findings:
"Material from trachea, containing granulation tissue with richly vascularised uncompact stroma and ***abundant presence of plasma cells but also acute inflammatory cells****. No signs of malignancy in the analyzed material.* ***In conclusion, the picture is consistent with granulation tissue."***
(Dec 20, 2011, Biopsy report, Appendix 18).
(Dec 20, 2011, Bronchoscopy Film 3 on the USB device).

Comments:
The statement of an bioartificial nanocomposite *"lined with a vascularised neomucosa, and was partly covered by nearly healthy epithelium"* are not supported by the biopsy findings of abundant presence of plasma cells, acute inflammatory cells and a picture consistent with granulation tissue. Lack of a vascularised neomucosa and healthy epithelium at 6 ½ months makes the presence of such at 5 months unlikely.

2. In the last sentence in the <u>*Methods section*</u> under the title <u>*The Recipient*</u> on p. 1998 the following statement is made: ***"We obtained written informed consent from the patient, and the transplant procedure was approved by the local scientific ethics committee."***

   Comments:
   Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board, which if confirmed, indicates that the above mentioned statement is a fabrication and potential violation against Swedish Law and the Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects (see General Comments).

3. In the <u>*Result section*</u> on p. 1999 the following statements can be found:
   "1 week after surgery, the bronchoscopy *(webvideo 3, figure 2A) showed a normal and patent airway bleeding from its inner layer at the contact with the scope; the obtained biopsy samples showed the presence of necrotic connective tissue associated with fungi contamination and neoformed vessels (figure 2B). The temporary tracheotomy cannula was removed 18 days later. The patient was then transferred to a normal ward and discharged to the referral hospital 1 month after surgery.* ***The biopsy sample 2 months after transplantation*** *showed large granulation areas* ***with initial signs of epithelialization and more organised vessel formations,*** *and* ***no bacterial or fungi contamination*** *(figure 2B). The patient was discharged from the referring hospital to start rehabilitation and later resumed his university studies.* ***5 months after transplantation, the patient is asymptomatic, breathes normally, is tumour free, and has an almost normal airway*** *(figure 2C) and improved lung function compared with preoperatively."*

   - Concerning the ***"1 week[1] after surgery"-***findings:
     No histological analyses are documented *"1 week after surgery"* in the Karolinska University Hospital medical records. According to the medical records the first biopsy is registered on Aug 4, 2011 (8 weeks after transplantation). There is no information in the Karolinska University Hospital medical records regarding analyses performed outside Karolinska and, if this is the case, where or by whom they were analyzed.
     [1]Approx. Jun 16, 2011.

     Subsequently, the statement referring to the biopsy findings 1 week after surgery is inconsistent since no biopsy has been performed at this time point.

- Concerning biopsy findings supporting the stated ***"2 months[2] after transplantation"***, the following biopsies have been registered in the Karolinska University Hospital medical records:
  [2]Approx. Aug 9, 2011.

  a. **Biopsy report from Aug 4, 2011 (8 weeks after transplantation) describes:**
  The nature of the sample: *"Synthetic trachea with cultured autologic cells"*
  Diagnostic Query: *"Structural overview of the synthetic graft, extracellular matrix proteins? Engrafted cells?"*
  The biopsy report describes:
  "In the sections from the three samples of the synthetic trachea which represent the left bronchus, right bronchus and trachea a similar picture ***of non-stainable porous material*** *with double refractory characteristics.* ***On the surface of this synthetic material only a few thin mesenchymal cells can be suspected. No well-developed cell layer could be identified."***
  (Aug 4, 2011, Biopsy report 8 weeks after transplantation, Appendix 7).

  Comments:
  No signs of initial epithelialization and vessel formation are mentioned in the medical records.

  b. **New Biopsies were taken again 12 days later on Aug 16, 2011 (10 weeks after transplantation) and revealed:**
  The nature of the sample: *"Three biopsies from a transplanted trachea"*
  Diagnostic Query: *"A frozen block shall be sliced and analyzed. The two other blocks shall just be sliced and stored at -80 Celsius."*
  "These biopsies are from the same graft (synthetic trachea) which was implanted in the patient in June this year."
  The biopsy report describes:
  "In the sections from the submitted samples can be found a cylinder of tissue that is composed of eosinophilic material similar to ***degenerated connective tissue with granulocytic reaction at the edge of the biopsy.*** *Using double refractory microscopy, collagen fibers can be detected. Even trichrome staining shows collagen fibers****. No intact nuclear staining, which implies advanced degeneration-necrosis.*** *Focally, basophilic granulocytic material can be identified. This can represent dystrophic calcification. Trichrome staining shows erythrocytes partially in seemingly shadow formations of vascular structures partially assumed in the interstitium.* ***PAS staining identifies fungal hypha****.* ***Gram-staining shows bacteria colonization."***
  (Aug 16, 2011, Biopsy report, 10 weeks after transplantation, Appendix 8a).

  The pathologist then performed a complementary examination on the Aug 16, 2011 on two other frozen biopsies taken at the same time with the following description:
  "*The other two frozen biopsies are also fixated and sectioned. One of them shows a similar picture of necrotic connective tissue with detectable fungal hyfa like the one above. The other one consists of capillary rich granulation, partially with an ulcerated surface, partially with recognizable epithelial lining showing squamous epithelial metaplasia."*
  Final diagnosis: ***"Biopsies from transplanted trachea with necrotic connective tissue with fungus and bacteria and capillary rich granulation."***
  (26 Aug, 2011, Complementary Biopsy report, Appendix 8b).

  Comments:

Advanced degeneration-necrosis, granulocytic reaction and the presence of fungi and bacteria contradicts the authors stated findings of "*initial signs of epithelialization and more organised vessel formations, and no bacterial or fungi contamination*".

c. **Additional Biopsies were repeated 8 days later on Aug 24, 2011 (11 weeks after transplantation) and revealed:**
The nature of the sample: *"Synthetic tracheal graft"*
Diagnostic Query: *"Cell ingrowth? Extracellular matrix structure?*
The biopsy report describes:
"In the sections from the four delivered small tissue samples a porous foreign material of synthetic graft can be identified. ***Detectable cellular components or matrix structures are not seen."***
(Aug 24, 2011, Biopsy report, 11 weeks after transplantation, Appendix 19).

Comments:
This contradicts the described *"initial signs of epithelialization and more organised vessel formations."*

4. In the *Results section* on p. 1999 the following statement can be found:
*"The patient was discharged from the referring hospital to start rehabilitation and later resumed his university studies****. 5 months[1] after transplantation****, the patient is asymptomatic, breathes normally, is tumor free, and has an almost normal airway** (figure 2C) and improved lung function compared with preoperatively."*
[12]Approx. beginning of Nov 2011.

Comments:
There are no records of any biopsies performed at the 5 months time point in the Karolinska University Hospital medical records but the biopsy findings from Aug 4, 16, and 24 (Appendix 7, 8a, 19) and the findings from Dec 20, 2011 (Appendix 20), all show a pathological and not an *"almost normal airway"* 2, 3 and 6½ months after transplantation, respectively. These essential findings should have been immediately reported to the Lancet Editor in order to correct the manuscript as the bronchoscopy findings were known before publication.

5. In the *Discussion section* on p. 2002 it is stated:
"In this report, an avascularised, Y-shaped nanocomposite was implanted and the initial fungal infection had resolved within ***4 months[1]*** *from transplantation; later the* ***endoluminal surface was partly lined with respiratory mucosa, at which we noted nearly healthy epithelium and proliferating endothelium.*** *This finding provides evidence that a bioengineered synthetic tracheobronchial nanocomposite can be recellularised in vivo with site-specific cells to become a living and functional scaffold completely integrated into the adjacent tissues. The measured levels of miR-34/449 micro-RNAs, which have been proposed as potential biomarkers of terminal differentiation of airway epithelium, [10] suggest the presence of postoperative airway epithelial differentiation in the patient."*
[1] Approximately beginning of Oct 2011.

Comments:
There are no ***"4 months"-***findings recorded in the Karolinska University Hospital medical records that can verify this statement. A *"endoluminal surface partly lined with respiratory mucosa"* or *"healthy epithelium and proliferating endothelium"* was not found in any of the biopsies performed 2-2½ months (Aug 4, 16 and 24, 2011) or at 6½ months (Dec 20, 2011, Appendix 20) after transplantation. The statement is also contradicted by the bronchoscopic findings 5½ months after transplantation (Nov 21 and 22, 2011, Appendix 16, 17 and Bronchoscopic Films 1 and 2 on the USB device).

Subsequently, the statement referring to the findings at *"4 months from transplantation…"* must until further be recognized as inconsistent with the information registered in the Karolinska University Hospital medical records.

6. Further down in the *Discussion section* the following conclusions are drawn on p. 2002:
   "This finding provides evidence that a bioengineered synthetic tracheobronchial nanocomposite can be recellularised in vivo with site-specific cells to become a living and functional scaffold completely integrated into the adjacent tissues."

   Comments:
   This conclusion is not supported by any bronchoscopic or biopsy data registered in the medical records. The information in the biopsy reports, the bronchoscopic findings and the clinical patient status must without any doubt have been fully known by the main author (Prof Macchiarini) *before* publication of the article on Nov 24, 2011, since he was present at the ENT-Department at that time (according to the medical records Nov 21, 2011, Appendix 16).

   In summary, in the registered data there is no evidence that the synthetic trachea is *"a living and functional scaffold"* at the time of publication. On the contrary, the bronchoscopic and biopsy findings indicate instead crucial and significant pathology as pointed out above.

7. Towards the end of the *Discussion section* the following conclusion is drawn on p.2003:
   "Taken together, these results provide evidence that a successful organ regeneration strategy has been accomplished (panel). The successful overall clinical outcome of this first-in-man bioengineered artificial tracheobronchial transplantation provides ongoing proof of the viability of this approach, *in which a cell-seeded synthetic graft is fabricated to patient-specific anatomical requirements and incubated to maturity within the environment of a bioreactor."*

   Comments:
   The bronchoscopic, biopsy and clinical admission data in the Karolinska medical records do not support this conclusion. The patient was readmitted with productive sputa, no respiratory sounds on the right chest wall, anastomotic granulations and a fistulation requiring bilateral stenting therapy (left and right main bronchus) *before* the article was published Nov 24, 2011.

General Comments:

1. The most severe inconsistency is that *no application for synthetic tracheal transplantation has been filed at the Regional Ethical Review Board* despite what the authors state in the article in the section titled The Recipient p. 1998.
   If this is confirmed, it may be a violation of:

   a. Swedish Laws and Regulations on Ethical Vetting of Research Involving Humans (Ethical Review Act, Svensk Författningssamling 2003:460, Statute Jan 1, 2004, Amendment 2007/08:44, Statute Jun 1, 2008).
   b. Swedish Medical Devices and Pharmaceuticals Acts (Medical Product Agency, Läkemedelsverket).
   c. The Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects.

2. No ethical application seems to have been filed at the Ethical Review Board but there is an *informed consent form* dated Jun 26, 2011 (signed by the investigator and the patient) registered in the Karolinska Medical records (Appendix 5a, b).
Specific comments concerning the attached informed consent form from Jun 26, 2011:

   a. First, the main objection against this informed consent form is that it **was signed on Jun 26, 2011, 17 days *after* the transplantation (Jun 9, 2011)**, which is a clear violation against the informed consent guidelines (The U.S Food and Drug Administration, among other Key organizations) which states that consent is to be documented ***before*** the planned intervention not after. Interestingly, the name of the scanned file of the consent form is labeled in the medical records "2011-05-26". (Surgical notes, June 9, 2011, Appendix 6)

   b. Second, an informed consent form before a highly invasive procedure as in this proof-of-concept-study cannot replace or substitute an ethical vetting as the informed consent form also has to be approved as a part of the study to prohibit manipulation or coercion of a desperate patient.

   c. Third, in the unapproved but present informed consent form there are several violations against the established guidelines on how to write a informed consent form recommended by The Swedish Central Ethical Review Board (Etiska Prövningsnämnden, EPN), FDA and other Key organizations:

      - 2nd part, 3rd line: *"…its reconstruction with a synthetic polymer-based and **completely biocompatible tracheal scaffold…"***

        Comments:
        "Biocompatibility" in this context can mislead the patient to believe that this synthetic material has been tested *in vivo* for this purpose and in this anatomical position, and thereby will be accepted by the surrounding native tissue. This was not scientifically proven at the time.

      - 3rd part, 1st line: *"I have read as well the protocol of the transplant procedure, written in English and **understand that this represents the only chance of survival I have."***

        Comments:
        This is a severe violation against the informed consent form guidelines stated by the FDA: *"Consent documents should not contain unproven claims of effectiveness or certainty of benefit, either explicit or implicit, that may unduly influence potential subjects.* *Overly optimistic representations are misleading and violate FDA regulations concerning the promotion of investigational drugs [21 CFR 312.7] or investigational devices [21 CFR 812.7(d)] as well as the requirement to minimize the possibility of coercion or undue influence [21 CFR 50.20]."*

      It would have been more realistic and honest to inform the patient that this was a experimental intervention with a material used for the first time as a trachea in a human being with no previous presented *in-vivo* animal data.

      - **No registered information is given about alternative interventions and no pros and cons are laid out** as suggested in the guidelines by the FDA: "… *subjects should be aware of the full range of options available to them.*

Consent documents should briefly explain any pertinent alternatives to entering the study including, when appropriate, the alternative of supportive care with no additional disease-directed therapy."

- **<u>The informed consent form is written in 1st person</u>,** which is clearly against the above mentioned FDA guidelines as:
  "Although not prohibited by the FDA regulations, use of the wording, "I understand..." in informed consent documents may be inappropriate as many prospective subjects will not "understand" the scientific and medical significance of all the statements. ***Consent documents are more understandable if they are written just as the clinical investigator would give an oral explanation to the subject, that is, the subject is addressed as "you" and the clinical investigator as "I/we."*** *This second person writing style also helps to communicate that there is a choice to be made by the prospective subject.* ***Use of first person may be interpreted as presumption of subject consent, i.e., the subject has no choice. Also, the tone of the first person "I understand" style seems to misplace emphasis on legal statements rather than on explanatory wording enhancing the subject's comprehension."***

  "Subjects are ***not in a position to judge whether the information provided is complete.*** *Subjects may certify that they understand the statements in the consent document and are satisfied with the explanation provided by the consent process (e.g., "I understand the statements in this informed consent document)." They should not be required to certify completeness of disclosure (e.g., "This study has been fully explained to me," or, "I fully understand the study.")".*

In conclusion, an incorrectly formulated and unapproved informed consent was signed 17 days after weeks after an invasive, unique and highly experimental intervention lacking ethical approval.

3. The "proof-of-concept study" is the most important of the articles reviewed in this analysis as it was the first one published (but not submitted) describing the "*first-in-man bioengineered artificial tracheobronchial transplantation"* and is used as a reference in the other articles that were published afterwards.

4. Three days *before* the online publication on Nov 24, 2011, the patient who is described as a success in the article, is readmitted looking *"a bit worn out"* with *"productive sputa", "no respiratory sounds on the right chest wall"* and having *"lost 7kg after the operation[1]".* The two bronchoscopies performed on Nov 21 and 22, 2011, confirmed a left sided fistulation, anastomotic stenoses secondary to granulations causing the right bronchus to be *"filled with pus*". The crucial bronchoscopic findings with need for interventions including insertion of 2 stents and no verification of transplant epithelialization are not consistent with the terminology *"asymptomatic"* and *"an almost normal airway.*" At this point it would have been possible to retract or postpone and adjust the publication.
   [1]Refers to the transplantation on Jun 9, 2011.

5. The bronchoscopy findings, stent interventions and clinical admission status must without any doubt have been known by Prof Macchiarini who was present at the ENT-Department at the time (according to the medical records from Nov 21, 2011, Appendix 16).

6. The present article demonstrates systematic presentation of inconsistent or fabricated clinical, bronchoscopic and histological findings together with omission of well known and registered data in the Karolinska University Hospital medical records.

Article 2

Engineered whole organs and complex tissues.

Badylak SF, Weiss DJ, Caplan A, Macchiarini P. *Lancet.* 2012 Mar 10; 379(9819):943-52. Review.
(Appendix 10).

Submitted Aug 12, 2011, published online Mar 10, 2012.

Major Inconsistencies:

1. Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board.

2. Unapproved informed consent form signed 17 days after the transplantation.

3. Fabrication and omission of biopsy findings.

4. Fabrication and omission of bronchoscopic findings.

5. Fabrication and omission of clinical status.

Inconsistent and Omitted Clinical Information:

1. In the section *Organ-specific examples* under the section *Respiratory system* on p. 946, it is stated:" *The artificial scaffold, which was seeded ex vivo with autologous bone marrow-derived stromal cells (in a bioreactor) and conditioned with pharmacological therapy, was implanted into a patient[1] with a primary recurrent*

tracheobronchial tumour. ***The graft was patent, well vascularised, and lined with a well-developed healthy mucosa 8 months[2] after transplantation.[3]"***

[1]Refers to Case 1 who was transplanted on Jun 9, 2011 and died at Karolinska on Jan 30, 2014.
[2]Approx. beginning of Feb 2012.
[3]Refers to the article: Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. Jungebluth P, Macchiarini P et al. *Lancet*. 2011 378:1997-2004. Epub 2011 Nov 24.
Comments:
The statement above refers to the *Lancet* article *"**Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study",*** Jungebluth P, Macchiarini P et al. published Nov 24, 2011, where *"5 months after transplantation data"* is presented (p. 1999). Using the "proof-of-concept study" which presents 5 months data as a reference to present 8 months data is an incorrect utilization of a reference and gives the impression of a longer follow-up than actually can be stated. It has also to be pointed out that the 5 months data referred to in the "proof-of-concept study" do not correlate to the registered findings in the medical records at the time. To be precise, the presented 5 month data referred to in the "proof-of-concept-study" should also more correctly have been presented as 4 month data considering the time interval from transplantation Jun 9 and submission of the "proof-of-concept-study" on Oct 11, 2011.

Furthermore, despite the above mentioned inconsistencies regarding the actual follow-up times the author uses the time point *"8 months"* in this publication omitting data that obviously became apparent *after* the publication of the "proof-of-concept study" on Nov 24, 2011.

This way of referencing gives the impression of the same "positive" outcome in the present "Engineered whole organs"-article as in the previous "proof-of-concept"-reference article, despite the crucial and negative findings that were diagnosed and treated ***before*** submission (Nov 21-22, 2011, Appendix 14, 15, 17) as well as ***after*** (Dec 20, 2011, Appendix 20) the publication of the "proof-of-concept-study" on Nov 24, 2011. Importantly, these results were well known and documented *before* the publication of the "Engineered whole organs"-article on Mar 10, 2012.

According to the *Lancet* (Editorial Assistant H. Baker on Jul 9, 2014) the reference article "proof-of-concept study" was submitted on Oct 11, 2011 but the "Engineered whole organs"-article was submitted on Aug 12, 2011, 9 weeks ***before*** the "proof-of-concept study". According to the same *Lancet* Editorial Assistant (Jul 9, 2014) that was because the "proof-of-concept study" was handled as a "fast-track-publication"-article which resulted in a online publication already on Nov 24, 2011, just 2 months after submission. That was not the case with the "Engineered whole organs"-article, which took until Mar 10, 2012 to be published, 7 months after submission on Aug 14, 2011. This would give the following time plan:

a. "Engineered whole organs"-article is **submitted on Aug 12, 2012.**

b. The "proof-of-concept study" is **submitted 2 months later on Oct 11, 2011.**

c. The "proof-of-concept study" is **published on Nov 24, 2011** presenting *"5 months after transplantation"*-data (which as mentioned above, should have been presented as 4 months data as the transplantation took place on Jun 9, 2011 and the submission date was 4 months later on Oct 11, 2011). The clinical and transplant tracheal graft deteriorates with visualization of fistulation and need for

bilateral stenting on Nov 21-22, 2011, which is 3 days before the online publication of the "proof-of-concept study" on Nov 24, 2011. The main author (Prof Macchiarini) was present at the ENT-department (according to the medical records Nov 21, 2011, Appendix 16) and must thereby have been fully informed about these crucial findings, however, no supplemental information was communicated to the *Lancet* regarding these important pre-publication findings.

d. "Engineered whole organs"-article is then **published on Mar 10, 2012,** 3½ months after the publication of the "proof-of-concept study" on Nov 24, 2011, stating the ***"graft was patent, well vascularised, and lined with a well-developed healthy mucosa 8 months after transplantation".***

2. On what is the statement "*8 months after transplantation"* based? The Karolinska medical records have registered 1 bronchoscopy, 2 biopsies and 1 brush sample from that time period, all were taken on Feb 14, 2012, which corresponds to 8 months after transplantation.

   Compared with the statement in the article the bronchoscopy, biopsy and brush sample reports describe:

   a. **Bronchoscopy on Feb 14, 2012, which is:**
      8 months ***after*** the transplantation on Jun 9, 2011.
      6 months ***after*** submission of the article on Aug 14, 2011.
      13 weeks ***after*** publication of the "proof-of-concept study" on Nov 24, 2011.
      13 weeks ***after*** the crucial bronchoscopic findings on Nov 21-22, 2011 (Appendix 15, 17).
      9 weeks ***after*** the bronchoscopic findings on Dec 20, 2011 (Appendix 20) and
      3 weeks ***before*** publication of the article on Mar 10, 2012.

      The bronchoscopy report describes:
      2nd line: *"Entering trachea and identify the anastomosis between trachea and the upper part o the graft. We see moderate with granulations at right and left. Passing further down and one can see* ***that the left of the two stents which previously have been inserted has migrated up and covers the right main bronchus departure."***
      11th line: "*Can see that there is a suspected* ***fistulation at 3-4 o´clock position, which also has been seen in this area previously.*** *There are some granulations here and they are removed. There is especially a granulation laterally left at 7-9 o´clock position which is removed."*
      Both stents are extracted and biopsies are taken.
      (Feb 14, 2012, Bronchoscopy report, Appendix 54).
      (Feb 14, 2012, Bronchoscopy Film 4 on the USB device).

      Comments:
      The statement *"patent graft lined with a well-developed healthy mucosa 8 months after transplantation"* is a gross misrepresentation and an example of data falsification. This is clearly demonstrated by the recorded bronchoscopies omitting the actual status of the transplant. Prof Macchiarini was present at the bronchoscopy on Feb 14, 2012 and must thereby have been fully aware of the bronchoscopic findings and interventions.

      2 new stents are subsequently re-inserted on May 22, 2012.
      (May 22, 2012, Bronchoscopy report, Appendix 41).
      (May 22, 2012, Bronchoscopy Film 11 on the USB device).

   b. **Biopsies 8 months after transplantation (Feb 14, 2012):**

The nature of the sample: *"Biopsies from the left main bronchus and from the trachea"*
Diagnostic Query: *"Granulation tissue? Mucoepidermoid cancer?*
The report describes:
"Biopsies from left main bronchus as well as trachea. In both fractions one can see a lot of granulation tissue with some plasmocyte infiltration. The surface epithelium consists partially of squamous epithelium which is eroded by granulocytic attack, partially completely rejected with scab formation, and focally single atypical squamous epithelial cells are seen but these seem to be mostly of a reactive character."
(Feb 14, 2012, Biopsy report, Appendix 21).

Comments:
Nothing in this biopsy report supports the statement *"lined with a well-developed healthy mucosa 8 months after transplantation."*

c. **Brush sample 8 months after transplantation (Feb 14, 2012) for cytological analysis of the types of cells growing in the graft:**
The nature of the sample: *"Brush sample from the lumen in the middle of the graft in trachea."*
Diagnostic Query: *"Epithelial finding? Airway epithelium? Other?*
The report describes:
"In the centrifuged fluid of delivered brush sample fluid, ***no significant epithelial material can be found."***
(Feb 14, 2012, Cytology brush sample report, Appendix 22).

Comments:
This finding contradicts the statement of a "*well-developed healthy mucosa 8 months after transplantation" .* A normal mucosa should yield normal (airway) epithelial cells upon brush sampling.

General Comments:

1. The previous "proof-of-concept-study" and the present "Engineered-whole-organs"-publication both demonstrate multiple examples of systematic omission, rephrasing and falsification of important registered findings (presence of significant granulations, fistulation, need for multiple stenting and no verification of airway graft re-epithelialization). All the above findings were well documented weeks and months in advance of the "Engineered whole organs"-publication, apparently without communicating this to the *Lancet* editors so the results could have been adjusted before publication and thereby presented to the medical community in congruence with the documented records.

2. There doesn't seem to be any ethical application or valid informed consent filed at the Review Ethical Review Board. If confirmed, this may be a severe violation against:

   a. Swedish Laws and Regulations on Ethical Vetting of Research Involving Humans (Ethical Review Act, Svensk Författningssamling 2003:460, Statute Jan 1, 2004, Amendment 2007/08:44, Statute Jun 1, 2008).
   b. Swedish Medical Devices and Pharmaceuticals Acts (Medical Product Agency, Läkemedelsverket).
   c. The Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects.

Article 3

Verification of cell viability in bioengineered tissues and organs before clinical transplantation.

Jungebluth P, Haag JC, Lim ML, Lemon G, Sjöqvist S, Gustafsson Y, Ajalloueian F, Gilevich I, Simonson OE, Grinnemo KH, Corbascio M, Baiguera S, Del Gaudio C, Strömblad S, Macchiarini P. *Biomaterials*. 2013 May; 34(16):4057 2013.02.057. Epub 2013 Mar 6. (Appendix 9).

Submitted Feb 5, 2013, published online Mar 6, 2013.

Major Inconsistencies:

1. Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board.

2. No informed consent form has been found in the medical records.

3. Fabrication and omission of biopsy findings.

4. Fabrication and omission of bronchoscopic findings.

5. Fabrication and omission of clinical status.

Inconsistent and Omitted Clinical Information:

1. In the *Results section,* on p. 4059, part 3.4 "A translational approach, development of a clinical tracheal graft", it is stated that: ***"A 21-year-old female patient[1]*** *suffered from an iatrogenic induced severe tracheal damage that affected the entire organ. An* immediate *transplantation was necessary to replace the entire trachea with a synthetic based TE tracheal graft. This surgery took place in August 2012 at the Department of Cardiothoracic Surgery and Anesthesiology at the Karolinska University Hospital, Stockholm (Sweden)."*

   [1]Refers to Case 3 who was transplanted on Aug 7, 2012 and re-transplanted on Jul 9, 2013, and still (August 2014) hospitalized in the Thoracic Intensive Care Unit at the Department of Cardiothoracic Surgery and Anesthesiology, Karolinska University Hospital, Solna, Sweden.

   Further down in the same *Results section* on p. 4065 it is stated that: "*The early clinical evaluation revealed an* ***initial graft epithelialization as judged from the 1-week post-operative brushing*** *(Fig. 7C). The intermediate post-operative outcome* ***(5 months[2]) has shown a patent and non-contaminated graft without any signs of inflammation…***"
   [2]Approx. beginning of January 2013.

   - Concerning the ***"1 week[1] post operative"-***findings:

     There is no record of a *"1-week post-operative brushing"* or a histological analysis to be found in the Karolinska University Hospital medical records. There is a note from Aug 14, 2012 (1 week after the transplantation):

*"Bronchoscopy by **Dr Macchiarini who thinks that it looks** very good in the graft and also down below. Little bit of mucous but no large amounts. Cultures are taken."*
[1]Approx. beginning of January 2013.
(Aug 14, 2012, Medical record note, Appendix 23).

Comments:
A subjective visual evaluation through a bronchoscope without biopsies doesn´t objectively (histologically) confirm if there is an initial graft epithelialization or not.

Totally 2 cytological analyses have been registered in the Karolinska University Hospital medical records until submission of the article on Feb 5, 2013 (6 months after the 1st transplantation Aug 7, 2012):

a. One pre-transplant analysis of the pulmectomized lung, Jul 24, 2012 (operation date for the pre-transplant right sided pulmectomy, not the 1st tracheal transplantation which was performed 2 weeks later, on Aug 7, 2012).

b. One biopsy from the tracheal graft 3 months (Oct 31, 2012) after the 1st transplantation (Aug 7, 2012):
The nature of the sample: *"Synthetic made trachea"*
Diagnostic Query: *"Viable cells? Respiratory epithelium?*
The report describes:
*"Minimal flakes of material reminds of **completely degenerated squamous epithelial cells infiltrated with a lot of bacteria** and single inflammatory cells. **The cells** do **not have a viable appearance.** Diagnosis: **non-viable squamous epithelial-like cells."***
(Oct 31, 2012, Biopsy report, Appendix 24).

Comments:
Even if 2 months before the stated *"5 months"* in the article, this is the only biopsy registered concerning the 1st transplant that this patient received and these findings do not support the statement "*patent and non-contaminated graft without any signs of inflammation…*" which also is clearly contradicted by the recorded bronchoscopy 6 months *after* transplantation and 2 weeks *before* submission of the article on Feb 5, 2013
(Jan 22, 2013, Bronchoscopy Film 5 on the USB device).

- Concerning the postoperative ***"5-months"-***statements in the article:

  There are no biopsies or bronchoscopies registered in the medical records after 5 months that support the statement in the article that **"*The intermediate post-operative outcome (5 months) has shown a patent and non-contaminated graft without any signs of inflammation…*"** but there are bronchoscopies recorded in Dec, 2012 (4½ months after the 1st transplantation) which document significant granulations, presence of stents and a fully established fistula.

  Prof Macchiarini was present at one of the bronchoscopies (Dec 18, 2012) and thereby fully informed about the bronchoscopic picture 7 weeks *before* submission of the article on Feb 5, 2013 (Appendix 25).

Additional clinical facts documented in the Karolinska medical records, concerning Case 3 until submission of article 3 (Feb 5, 2013) 6 months after the 1st transplantation (7 Aug, 2012):

1. **Diagnosis of the tracheoesophageal fistulation (TEF) 17 days (Aug 24, 2012)** after the 1st transplantation: *"The patient has a diagnosed fistulation between the trachea and esophagus, probably at the distal anastomotic level.* ***Discussion with Macchiarini and E.J. about how to handle this."***
   (Aug 24, 2012, Medical record note, Appendix 26).

2. **Gastroscopy 18 days (Aug 25, 2012) after the 1st transplantation with insertion of the 1st esophageal stent and percutaneous gastrostomy (PEG) in order to unload the TEF.**
   The operation report describes:
   6th line *"At ca. 25 cm from the tooth row one sees a ulceration with a small opening probably to the trachea."*

   16th line: *"Choosing to insert a fully coated Wallflex stent 155mm long and 28mm in diameter at the collars."*
   (Aug 25, 2012, Operation report, Appendix 27).

3. **Bronchoscopy 3 weeks (Aug 29, 2012) after the 1st transplantation with insertion of the 1st tracheal and left main bronchus stents (2 stents) due to a compressed scaffold.**
   The operation report describes:
   21st line: "*Thus, there are granulations in the lower anastomosis in the carina level, especially on the dorsal side and inwards to carina. After clean suction,* ***one stent is first inserted,*** *Ultraflex size 18mm in diameter, 40mm in length…", "…the lower edge of the stent reaches precisely the suture line."*
   24th line: *"Thereafter is a* ***further stent inserted*** *which again is a Ultraflex and this is a bronchial stent it is 14mm in diameter and 30 mm in length…"*
   (Aug 29, 2012, Operation report, Appendix 29).

4. **Bronchoscopy 3½ weeks (Aug 31, 2012) after the 1st transplantation for re-stenting of the esophagus after the previous airway stenting to accomplish a *re-double stenting* of the airway/esophagus.**
   The operation report describes:
   1st line: *"Gastroscopy down the esophagus where you at 20cm have a bloated mucus membrane in the frontal wall with some secretion which probably represents the fistulation."*
   4th line: *"Insert a Savary guide and insert a Wallflex 23mm x 125mm fully coated* ***esophageal stent…"***
   (Aug 31, 2012, Bronchoscopy report, Appendix 28).

5. **Bronchoscopy film 6 weeks (Sep 18, 2012) after the 1st transplantation** clearly showing increased secretion originating from the fistulation.
   (Sep 18, 2012, Bronchoscopy Film 6 on the USB device).

6. **Bronchoscopy film 7 weeks (Sep 26, 2012) after the 1st transplantation** clearly showing fistulation.
   (Sep 26, 2012, Bronchoscopy Film 7 on the USB device).

7. **Bronchoscopy 10 weeks (16 Oct, 2012)** after the 1st transplantation.
   The operation report describes:
   4th line: ***"At 7 o´clock position we see an un-irritated rather large fistula opening which opens when the ventilator delivers a breath.*** *Completely un-irritated mucus membrane in the fistulation channel which I think could be intubated with the bronchoscope, but the stents are too close to each other preventing this. The fistulation channel goes down and clearly to the right. "*

(Oct 16, 2012, Operation report, Appendix 30).

8. **Bronchoscopy 10 weeks (Oct 18, 2012)** after the 1st transplantation due to repeating *"voluminous regurgitations from the esophagus into the trachea. Stenting needed to cover between the two stent already in place".* Previous 2 stents are supplemented with a 3rd tracheal stent.
   The operation report describes:
   6th line: *"Insert an 18x400mm* ***tracheal stent within the present stents with good fit over the fistulation area."***
   (Oct 18, 2012, Operation report, Appendix 31).

9. **Bronchoscopy 3 months (Oct 31, 2012)** after the 1st transplantation with exchange of the previous 3 stents to 2 new stents due to *"…problems with a lot of accumulation of secretion in all stents."*
   The operation report describes:
   33rd line: ***"Has now 2 stents;*** *1. 14x300mm centrally coated stent in the left main bronchus. 2. 18x400mm centrally coated stent primarily in the bronchial stent but it reaches cranially and covers the anastomotic area and the suspected fistulation."*
   (Oct 31, 2012 Bronchoscopy report, Appendix 32).

   Comments.
   Prof Macchiarini was present at the bronchoscopy on Oct 31, 2012 and must thereby have been fully aware of the bronchoscopic findings and interventions.

10. **Bronchoscopy/gastroscopy 13 weeks (Nov 13, 2012)** after the 1st transplantation for fistula mapping reveals:
    6th line: *"Gastroscopy: While the gastroscopy is performed and is identifying the fistula region,* ***we can from the bronchial side, see the gastroscope pass the area of fistulation, the fistulation is obviously an anastomotic insufficiency, probably is it the continuous suture line in the membranous part that is gone as the whole posterior quadrant is a fistula opening."***
    46th line: ***"it is possible to enter the esophagus from the trachea with the bronchoscope without problems."***
    (Nov 13, 2012, Operation report, Appendix 33).

11. **Bronchoscopy 4½ months (Dec 19, 2012)** after the 1st transplantation with subacute exchange of the stents due to stent migration and increasing granulation tissue resulting in increased airway resistance.
    (Dec 19, 2012, Bronchoscopy report, Appendix 34).

12. **Bronchoscopy film 5½ months (Jan 22, 2013)** after the 1st transplantation.
    (Jan 22, 2013, Bronchoscopy Film 5 on the USB device).

Case 3 underwent in total 55 concomitant operations between Aug 7, 2012 and Feb 5, 2013 (submission of the present article).
The 8 operations from the initial tracheal resection and right-sided pulmectomy (Jul 24, 2012) until the tracheal transplantation 14 days later (Aug 7, 2012) are not included in that number.
(List of surgical procedures from Jul 25, 2012 to Aug 3, 2013, Appendix 35).

General Comments:

1. A fully stented graft covered with minimal flakes of non-viable squamous epithelial-like cells and a lots of bacteria, a fully established tracheo-esophageal fistulation and total dependence on multiple daily bronchoscopies because otherwise resulting aspiration,

stent obstruction and suffocation is not consistent with the stated *"a patent and non-contaminated graft"*.

2. This article is further evidence of omission concerning clinically important, well-known and documented data correlated to the extremely tragic and troublesome postoperative situation that **Case 3 still endures since over 2 years of hospitalization in the ICU. The hospital stay has included ventilator therapy for 315 days, 3 times on ECMO-support (totally 72 days) and continuous renal replacement therapy for over 7 weeks because of the necessity of frequent reoperations and complications. Case 3 is still hospitalized even though she is fully mobilized, communicative and has scheduled bronchoscopies at least every 4th hour around the clock, 7 days a week and is subsequently chronically tracheostomized. This patient has been surgically mutilated requiring daily aspiration of pus from the operative chest- and esophagus rest-cavities maintained with 2 permanent chest drains through a persistent opening on the right chest wall, has chronic treatment with broad spectrum antibiotics and antifungal therapy, is nutriating through a percutaneous gastric tube due to the cervical esophagostomy which makes all the "comfort"-orally-swallowed food come out in a plastic bag on her neck.** She is often depressed as she has lost all quality of life compared with her state previous to the 1st transplantation but still hopes for a partial recovery.

3. In the *Result section* on p. 4059, part 3.4: *A translational approach, development of a clinical tracheal graft* it is stated that: *"An **immediate** transplantation was necessary to replace the entire trachea with a synthetic based TE tracheal graft."*

   The patient`s state at the time she was evaluated and offered a procedure by Prof Macchiarini more than 4 months (Istanbul Mar 24, 2012) *before* it finally took place in Stockholm Aug 7, 2012 was the following:

   Case 3 was living at home after she was released from the Istanbul Hospital in October 2011, 5 months before the meeting with Prof Macchiarini. The patient describes a moderate loss of physical performance since the hospital stay in Turkey (functionally pulmectomized on the right side) but her status was essentially stable since her release in October 2011. The patient could walk in a normal pace for 200-300 meters, climb two flights of stairs without resting, visit cafes, take the bus back and forth to the shopping mall, eat normally and had no dyspnoea or need for supplemental oxygen. However, she had a troublesome chronic cough that she describes as intermittent. During the daytime she started the morning with major coughing for several minutes ("cleaning" her airway, spitting out mucous) which then more or less stopped for 3-4 hours before a new coughing episode ensued. Usually she went to bed around 11-12 pm waking up at 1-2 am because of coughing and mucus production and then she could sleep the rest of the night through. She had a chronic Heimlich drainage placed into the right pleural cavity (inserted already back in Jul 25, 2011) through an opening into her right chest, connected to plastic bag which she changed by herself once every day (with 1-2 fingers width of secretion at its bottom) but she could take daily showers and manage her hygiene without assistance, covering the drainage with plastic. She was continuously followed up every 1-2 weeks with ***visits to an outpatient clinic (the patient was not hospitalized).***

   Meeting with Prof Macchiarini implied a domestic flight for one hour (from her hometown of Ordu to Istanbul). The patient was clinically evaluated and thereafter flew back home. The patient denies clearly that her physical status was deteriorating in the months prior to the preoperative evaluation or during the 4 months of "waiting time" before the transplantation finally took place in August, 4 months later.

(Jun 25, 2014, According to the patient's own words registered in the Medical Records).

To summarize, Case 3 had "*suffered from iatrogenic induced severe tracheal injury…"* but to claim that the patient was a candidate for an immediate intervention is highly questionable with the above described relatively stable functional degree without rapid deterioration.

4. The reason for using the term *"immediate"* by the authors despite the above described status can just be speculated upon, but it has to be taken in account that there doesn't seem to have been any application filed at the Review Ethical Review Board for this elective and experimental surgical approach despite that the decision for the intervention was taken over 4 months before the transplantation procedure, and which if confirmed, may be a severe violation against:

   a. Swedish Laws and Regulations on Ethical Vetting of Research Involving Humans (Ethical Review Act, Svensk Författningssamling 2003:460, Statute Jan 1, 2004, Amendment 2007/08:44, Statute Jun 1, 2008).
   b. Swedish Medical Devices and Pharmaceuticals Acts (Medical Product Agency, Läkemedelsverket).
   c. The Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects.

Article 4

Are synthetic scaffolds suitable for the development of clinical tissue-engineered tubular organs?

Del Gaudio C, Baiguera S, Ajalloueian F, Bianco A, Macchiarini P. *J Biomed Mater Res A*. 2014 Jul;102(7):2427-47. Epub 2013 Aug 2.
(Appendix 11).

Submitted Mar 18, 2013, published online Aug 2, 2013.

Major Inconsistencies:

1. Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board.

2. Unapproved informed consent form signed 17 days after the transplantation.

3. Serial fabrication and omission of biopsy findings.

4. Serial fabrication and omission of bronchoscopic findings.

5. Fabrication and omission of clinical status.

Inconsistent and Omitted Clinical Information:

The article states on p. 11 that *"A relevant and significant application of tissue-engineering concepts was clinically carried out by implanting a synthetic tracheal substitute into a **36-year old male patient**[1] affected by a recurrence of a primary tracheal mucoepidermoid carcinoma involving the distal trachea and both main bronchi.[44] ….**At 1 week**, postoperative bronchoscopy assessments verified a normal and patent airway, whereas biopsy samples showed the presence of necrotic connective tissue associated with fungi contamination and neovessels. **After 2 months** from transplantation, biopsy revealed large granulation areas associated with smooth epithelialization and some organized vessels formation while neither bacterial or fungi contamination was observed. **After 12 months**, an **almost normal airway** and improved lung function were assessed."*
[1]Refers to Case 1 who was transplanted on June 9, 2011. The patient died at Karolinska on Jan 30, 2014.

At 1 week[1] after transplantation the article states that: ***"…biopsy samples** showed the presence of necrotic connective tissue associated with fungi contamination and neovessels."*
[1]Approx. Jun 16, 2011.

Comments:
No post-transplantation analyses of biopsies are documented in the Karolinska University Hospital medical records until Aug 4, 2011 (8 weeks after transplantation on Jun 9, 2011) and there is no information in the Karolinska University Hospital medical records if there have been any analyses made outside Karolinska University Hospital. Subsequently, the statement referring to the stated biopsy findings are inconsistent since no biopsies have been registered at 1 week.

At 2 months[2] after transplantation the article on p. 11 states that: *"biopsy revealed large granulation areas associated with smooth epithelialization and some organized vessels formations while **neither** bacterial or fungi contamination was observed."*
[2]Approx. Aug 9, 2011

The following reports are recorded in the Karolinska University Hospital medical records:

1. **Biopsies 8 weeks after transplantation (Aug 4, 2011) revealed:**
   The nature of the sample: "*Synthetic trachea with cultured autologic cells"*

Diagnostic Query: *"Structural overview of the synthetic graft, extracellular matrix proteins? Engrafted cells?"*
The report describes:
"In the sections from the three samples of the synthetic trachea which represent the left bronchus, right bronchus and trachea a similar picture of non-stainable porous material with double refractory characteristics. On the surface of this synthetic material only a few thin mesenchymal cells can be suspected. ***No well-developed cell layer could be identified.****"*
(Aug 4, 2011, Biopsy report 8 weeks after transplantation, Appendix 7).

Comments:
"Smooth epithelialization" and vessel formation are not mentioned in this biopsy report.

2. **New Biopsies were taken 12 days later (Aug 16, 2011) 10 weeks after transplantation and revealed:**
   The nature of the sample: *"Three biopsies from a transplanted trachea"*
   Diagnostic Query: *"A frozen block shall be sliced and analyzed. The two other blocks shall just be sliced and stored in -80 Celsius."*
   "These biopsies are from the same graft (synthetic trachea) which was implanted in the patient in June this year."
   The report describes:
   "In the sections from the submitted samples can be found a cylinder of tissue that is composed of eosinophilic material similar to ***degenerated connective tissue with granulocytic reaction at the edge of the biopsy.*** *Using double refractory microscopy, collagen fibers can be detected. Even trichrome staining shows collagen fibers.* ***No intact nuclear staining, which implies advanced degeneration-necrosis****. Focally, basophilic granulocytic material can be identified. This can represent* ***dystrophic calcification.*** *Trichrome staining shows erythrocytes partially in shadow formations of vascular structures partially in the interstitium can be assumed. PAS staining* ***identifies fungal hypha. Gram-staining shows bacteria colonization."***
   (Aug 16, 2011, Biopsy report, 10 weeks after transplantation, Appendix 8a).

   The pathologist then performed a complementary examination on the Aug 16, 2011 on two other frozen biopsies taken at the same time with the following description:
   "*The other two frozen biopsies are also fixated and sectioned. One of them shows a similar picture of necrotic connective tissue with detectable fungal hyfa like the one above. The other one consists of capillary rich granulation, partially with an ulcerated surface, partially with recognizable epithelial lining showing squamous epithelial metaplasia."*
   Final diagnosis: ***"Biopsies from transplanted trachea with necrotic connective tissue with fungus and bacteria and capillary rich granulation."***
   (26 Aug, 2011, Complementary Biopsy report, Appendix 8b).

   Comments:
   The statement in the article is not supported by this biopsy report, which describes degenerated connective tissue with granulocytic reaction, advanced degeneration-necrosis and the presence of fungi and bacteria in the biopsies.

3. **New biopsies were taken another 8 days later (Aug 24, 2011) 11 weeks after transplantation and revealed:**
   The nature of the sample: *"Synthetic tracheal graft"*
   Diagnostic Query: *"Cell ingrowth? Extracellular matrix structure?*
   The report describes:

"In the sections from the four delivered small tissue samples a porous foreign material of synthetic graft can be identified. ***Detectable cellular components or matrix structures are not seen."***
(Aug 24, 2011, Biopsy report, 11 weeks after transplantation, Appendix 19).
Comments:
This is not the equivalent to the articles statement of smooth epithelialization and vessel formation.

The following findings are attached to illustrate the patient's clinical status between the **2nd and the 12th month** which are also the presented time points in the article.

1. **Bronchoscopy on Nov 21, 2011 (5½ months after transplantation) revealed:**
   4th line: *"there are distal granulations on the distal graft endings on both the right and left side. Starting by inspecting that there is a passage down in the left main bronchus which is stenosed but not more than to maybe 20%."*
   8th line: *"I first go down and recess the granulations on the right side in the main bronchus. It is possible to reduce them. They bleed fairly easily but it is possible to reduce the masses, it is not possible to clearly identify the branching of the upper lobe.*
   14th line: ***"Decide to implant a stent…"*** (Right main bronchus).
   19th line: *"One can suspect a* ***small opening at 1 o'clock position****, in other words inside medially in front exactly at the edge of the graft.* ***Implant even here a stent…"*** (Left main bronchus).
   (Nov 21, 2011, Bronchoscopy report, Appendix 15).
   (Nov 21, 2011, Bronchoscopy Film 1 on the USB device).

2. **Bronchoscopy was repeated the next day Nov 22, 2011, with the following findings:**
   4th line: *"…the right bronchus is filled with pus. It looks better on the left side. Sucking clean. It fills up all the time from down below on the right side."*
   12th line: **"…*serous fluid is entering through the anastomosis between graft and bronchus at 6-7 o´clock position, right side."***
   15th line: ***"…inserting a new stent…"*** (Right main bronchus).
   23rd line**: "*See fistulation which lies medially and at the edge of the graft, on this side at 2-3 o´clock position."*** (Left main bronchus).
   24th line: *"Finish by pulling the stent up again so that I achieve a nice cover in applied and treated surfaces"* (Left sided stent).
   (Nov 22, 2011, Bronchoscopy report, Appendix 17).
   (Nov 22, 2011, Bronchoscopy Film 2 on the USB device).
   Comments:
   Prof Macchiarini was present at the ENT-Department according to the medical records Nov 21, 2011, and must thereby have been fully aware of the bronchoscopic findings and interventions Nov 21-22, 2011.
   (Nov 21, 2011, Medical record note, Appendix 16).

   Significant granulations in the anastomotic regions, exchange of the right sided and repositioning of the left sided stent. The bronchoscopy findings clearly contradict the statements in the article of a *"patent" or "almost normal airway."*

3. **Bronchoscopy was repeated two weeks later on Dec 20, 2011 (6½ months after transplantation) and revealed:**
   7th line: ***"Between the graft and the bronchus[1] at 3-4 o'clock position a fistulation is seen."*** Bilateral stents are exchanged.
   [1]Left main bronchus
   (Dec 20, 2011, Bronchoscopy report, Appendix 20).

(Dec 20, 2011, Bronchoscopy Film 3 on the USB device).

4. **Biopsies on Dec 20, 2011 (6½ months after transplantation) revealed:**
   The nature of the sample: *"mucus membrane/granulation from the trachea"*
   Diagnostic Query: *"granulation? ca?"*
   The report describes:
   "Material from trachea, containing granulation tissue with richly vascularised uncompact stroma and abundant presence of plasma cells but also acute inflammatory cells. No signs of malignancy in the analyzed material. In conclusion, ***the picture is consistent with granulation tissue."***
   (Dec 20, 2011, Biopsy report, Appendix 18).

   Comments:
   Smooth epithelialization is not mentioned in this biopsy report.

5. **Bronchoscopy on Feb 14, 2012 (8 months after transplantation) which is:**
   13 months ***before*** submission of the article on Mar 18, 2013.
   12 weeks ***after*** the crucial bronchoscopic findings on Nov 21-22, 2011 (Appendix 15, 17).
   1½ year ***before*** publication of the article on Aug 2, 2013.

   The bronchoscopy report describes:
   2nd line: *"Entering trachea and identify the anastomosis between trachea and the upper part o the graft. We see moderate with granulations at right and left. Passing further down and one can see* ***that the left of the two stents which previously have been inserted has migrated up and covers the right main bronchus departure."***
   11th line: "*Can see that there is a suspected* ***fistulation at 3-4 o´clock position, which also has been seen in this area previously[1].*** *There are some granulations here and they are removed. There is especially a granulation laterally left at 7-9 o´clock position which is removed."*
   Both stents are extracted and biopsies are taken.
   (Feb 14, 2012, Bronchoscopy report, Appendix 54).
   (Feb 14, 2012, Bronchoscopy Film 4 on the USB device).

   Comments:
   The statement "*an almost normal airway"* is not supported by this bronchoscopy.
   The 2 extracted stents were subsequently replaced by 2 new stents on May 22, 2012.
   Prof Macchiarini was present at the bronchoscopy on Feb 14, 2012 and must thereby have been fully aware of the bronchoscopic findings and interventions.

6. **Biopsies on Feb 14, 2012 (8 months after transplantation) revealed:**
   The nature of the sample: *"Biopsies from the left main bronchus and from the trachea"*
   Diagnostic Query: *"Granulation tissue? Mucoepidermoid cancer???*
   The report describes:
   "Biopsies from left main bronchus as well as trachea. In both fractions one can see a lot of granulation tissue with some plasmocyte infiltration. The surface epithelium consists partially of squamous epithelium which is eroded by granulocytic attack, partially completely rejected with scab formation, and focally single atypical squamous epithelial cells are seen but these seem to be mostly of a reactive character."
   (Feb 14, 2012, Biopsy report, Appendix 21).
   (Feb 14, 2012, Bronchoscopy Film 4 on the USB device).

   Comments:

Nothing in this biopsy report supports the statement of *"an almost normal airway"* even if these biopsies were taken 8 months and not at 12 months after transplantation.

7. **Brush sample on Feb 14, 2012 (8 months after transplantation) for cytological analysis of the types of cells growing in the graft, revealed:**
   The nature of the sample: *"Brush sample from the lumen in the middle of the graft in trachea."*
   Diagnostic Query: *"Epithelial finding? Airway epithelium? Other?*
   The conclusion by the pathologist is the following:
   "In the centrifuged fluid of delivered brush sample fluid, no significant epithelial material can be found."
   (Feb 14, 2012, Cytology brush sample report, Appendix 22).
   Comments:
   This contradicts the statement of "s*mooth epithelialization".* A normal mucosa should yield normal epithelial cells upon brush sampling.

A t **12 months**[3] after transplantation the article on p. 11 states that: *"…an almost normal airway and improved lung function were assesse*d."
[3]Approx. 9 June 2012.

In the Karolinska University Hospital medical records the following findings are registered which are temporally closest to the ***"12 months"***- statement in the article of *"an almost normal airway":*

1. **Biopsies on May 22, 2012 (11 months after transplantation) revealed:**
   The nature of the sample: *"Sample containing biopsies from granulations right main bronchus and from tracheal graft in trachea.*"
   Diagnostic Query: *"Granulations? Signs of malignancy? Bronchial epithelium?"*
   ***"Box 2;** Granulations from the anastomosis in the right main bronchus and the tracheal anastomosis."*
   ***"Box 3;** biopsy from the left side above the area of carina in the middle of the graft."*
   The report describes:
   "II: Tissue samples containing squamous epithelium with pronounced and unspecific stromal inflammation. Significant eosinophilia. No malignancy."
   *"III: Having judged everything, **nothing but an acellular structure is seen**, residual components of the graft itself?"*
   (May 22, 2012, Biopsy report 11 months after transplantation, Appendix 40).

   Comments:
   Nothing in this biopsy report can support the article's statement of *"an almost normal airway".* There is granulation tissue at the right and proximal anastomoses and the graft itself shows nothing but acellular structures.

2. **Bronchoscopy on May 22, 2012 (11 months after transplantation) revealed:**
   1st line: "*The patient has **easy bleeding granulations mostly from 2 to 5 o´clock position and also 8 to 10-11 o'clock position thus on the left side at the upper anastomosis."***
   8th line: *"We see that there is **profuse secretion that occludes especially the right main bronchus opening…"***
   12th line: At the left main bronchus anastomosis: *"One can see a **fistula between the 2 o´clock and 3 o'clock position** where it also bubbles a little bit. It has been seen there for the last half-year, seems a little smaller today. One can even see, between the **1 o'clock and 3 o'clock positions, exposed cartilage, these are cartilage rings."***

16th line: ***"right bronchus…..severe granulation with stenosis of the lumen downstream to the degree that only the smallest suction tube can pass through, at largest 2mm in diameter on right side, distally."***
27th line: ***"implant stent*** *14 mm wide, 30 mm in length*" (in the right bronchus).
36th line: ***"1 suture has detached at 1-3 o´clock position*** *on the right distal anastomosis."*
(May 22, 2012, Bronchoscopy report 11 months after transplantation, Appendix 41).
(May 22, 2012, Bronchoscopy Film 11 on the USB device).

Comments:
These are crucial and contradictive findings compared with the statement in the article of *"an almost normal airway"*.

General Comments:

1. This article demonstrates systematic presentation of inconsistent clinical, bronchoscopic and histological findings together with omission of well known and registered data of importance in the Karolinska University Hospital medical records.

2. A non-epithelialized transplant showing nothing but an acellular structure, an established left sided fistulation known for 6 months, exposed left-sided cartilaginous rings, significant granulations in the anastomotic regions and the presence of 2 bronchial stents is not consistent with *"an almost normal airway…"*

3. Furthermore, no information about the patients´ continuously deteriorating status is presented in the article as described in the letter sent from the Department of Cardiothoracic Surgery in Reykjavik, Island to the Karolinska University Hospital, dated Aug 3, 2013:

   "For over a year[1] XX[2] has suffered bothering respiratory symptoms that have only got worse*. It started with* ***recurrent hemoptysis*** *that required admission to our hospital. It was thought that these bleedings were related to granulation tissue at the anastomotic sites but potentially also from the stents. After intermittent treatment with Cycklocapron and bronchoscopic controls in Stockholm these problems have got better. Instead,* ***recurrent infections, mainly in XX right lung, have been the main concern for the last 8 months****[3]. **In December 2012**[4] XX was diagnosed with a* ***rather large abscess*** *in XX right lower lobe that gradually responded to iv. antibiotics. Since then XX has been admitted* ***multiple times to our hospital for copious blood tinged sputum and pneumonia-like symptoms.*** *Streptomonas mult. bacteria have been grown from his trachea, but have been resistant to treatment."…*

   "Since early June[5] XX has been more in our hospital than at home. He is not septic but his problems with copious sputum and hemoptysis are worrisome and reduce his quality of life significantly."…

   "With multiple investigations we have shown that XX lung is non-functioning. This is due to an early postoperative thrombus of a Vascutec graft to the right pulm. artery and multiple distal embolies to the right lung. His right lung therefore seems to contribute minimally to his respiration."
   (Appendix 42).

   [1]Approx. summer 2012 which means at least 8 months before submission of the article on Mar 18, 2013.

[2]Refers to Case 1 who was transplanted on June 9, 2011. The patient died at Karolinska on Jan 30, 2014.
[3] "last 8 months" was approx. 3 months before submission of the article on Mar 18, 2013.
[4] "December 2012" is 2-3 months before submission of the article on Mar 18, 2013.
[5]June 2013.

The mentioned findings in the letter above were known, communicated and documented several months before submission of the article on Mar 18, 2013.

4. No ethical application for this procedure has been filed at the Review Ethical Review Board which if confirmed, may be a severe violation against:

    a. Swedish Laws and Regulations on Ethical Vetting of Research Involving Humans (Ethical Review Act, Svensk Författningssamling 2003:460, Statute Jan 1, 2004, Amendment 2007/08:44, Statute Jun 1, 2008).
    b. Swedish Medical Devices and Pharmaceuticals Acts (Medical Product Agency, Läkemedelsverket).
    c. The Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects.

Article 5

Airway transplantation.

Jungebluth P, Macchiarini P. *Thorac Surg Clin.* 2014 Feb; 24(1):97-106. Review. (Appendix 12).

Submitted Aug 4, 2013, published online Feb 24, 2014.

Major Inconsistencies:

1. Omission of biopsy findings.
2. Omission of bronchoscopic findings.
3. Omission of clinical status and outcome data.

Inconsistent and Omitted Clinical Information:

1. In Table 3 on p. 104 under the section *Synthetic-based Trachea (2011-2013)* under *Outcome* the statement is made: *"1 (out of 6) patient died of unrelated causes".*

   Comments:
   Before submission of this article on Aug 4, 2014, oral information was given by the research group of Prof Macchiarini that Case 2, who is referred to as dead, died in his resident country (USA) due to a fatal bleeding secondary to fistulation of the tracheal transplant, which led to aspiration of blood and suffocation. No documentation concerning cause of death is available in his Karolinska University Hospital medical records. No autopsy was performed according to the same research group. It is not possible to draw the conclusion or to prove that the patient died of *"unrelated causes"* 3½ months after a major surgical airway intervention involving implantation of a unique synthetic tracheal scaffold as an autopsy was never performed. On the contrary, it must be more reasonable to suspect that a previous major operation in the central airways involving vascular surgery of the large vessels to be a main factor involved in an acute massive and fatal airway bleeding 3½ months later (arterial-tracheo fistulation?).

2. In Table 3 on p. 104 under the section *Synthetic-based Trachea (2011-2013)* under *Outcome* it is further stated that: *"To date all patients are alive (only the POSS/PCU scaffold requires stent treatment because of abnormal granulation tissue and fistula formation)".*

   Comments:
   In two (Case 1 and Case 3 as referred to in the table above) of the three patients transplanted at Karolinska University Hospital, extensive and repeated airway stenting has been necessary. In Case 3 the previous tracheal transplant as well as the current (re-transplanted on 9 Jul, 2013, due to material fatigue and severe anastomotic fistulation) trachea transplant, have required repetitive and extensive

stenting. In all that makes three synthetic scaffolds in need of extensive stenting, not one. The extensive need for stenting was known before submission of this article on Aug 4, 2013.

(Nov 22, 2012, CT scans of Case 1, Left and right main bronchi are both stented. Upper right lobe shows signs of extensive consolidation, Appendix 43).
(Sep 6, 2012, CT scans of Case 3 with a stented synthetic trachea and a stent in the esophagus in an attempt to treat the tracheo-esophageal fistulation, CT scan was performed before the loop esophagostomy on Apr 9, 2013 and the later transhiatal esophagectomy which was performed on Aug 6, 2013. The submission of this article on Aug 4, 2013 was 11 months after the CT scan with visualized the double stenting and accordingly fully known by the authors at submission, Appendix 44).

General Comments:

1. No data is presented regarding the development of a large tracheo-esophageal fistula with the need for re-transplantation of a new synthetic trachea in Case 3. Re-transplantation was decided on May 8, 2013 with Prof Macchiarini present nearly 3 months *before* submission of the article on Aug 4, 2013.
   Tracheo-esophageal fistulation is a fatal condition where an opening between the trachea and the esophagus has developed which makes normal eating impossible and the risk for pneumonia and mediastinitis imminent when the airways gets contaminated with fluid/secretion from the intestinal tract.
   (Jul 9, 2013, Operation report by Prof Macchiarini on synthetic trachea re-transplantation, Appendix 45. Date of surgery was 26 days *before* submission of this article on Aug 4, 2013.

2. No data is presented regarding the need to unload (exclude) the esophagus in Case 3. The cervical loop esophagostomy with an esophageal opening on the neck was performed on Apr 9, 2013, 4 months before submission of the article on Aug 4, 2013. This had later to be followed by a full transhiatal esophagectomy. The date for deciding when to operate the patient's esophagus was Jul 30, 2013, five days before submission of the article and the operation took place on Aug 6, 2013 just 2 days after submission of the article (Aug 4, 2013) and subsequently, the esophagectomy was known by Prof Macchiarini at the time of submission on Aug 4, 2013.

3. No data is presented regarding the reoccurrence of the distal anastomotic fistula after the re-transplantation (Jul 9, 2013) of Case 3 which was diagnosed 14 days after the re-transplantation on Jul 23, 2013 and stented the day after on Jul 24, 2013.
   (Jul 23, 2013, CT scan report of Case 3, Appendix 46a and 46b, showing distal anastomotic fistula performed before submission date of the article on Aug 4, 2013, and the radiological description stating contrast leakage from esophagus to both the thoracic cavity and into the tracheal transplant, Appendix 47).

4. No data is presented regarding the number of, or development of scaffold material fatigue, which together with the tracheo-esophageal fistulation was the second major reason for the re-transplantation in Case 3, as the scaffold fully collapsed between January and March 2013 and had to be stented in its whole length not to obstruct the airway.

5. No data is presented regarding the number of, or need for re-transplantations. The research group of Prof Macchiarini gave the oral information before the submission of the article that one Russian female with the same scaffold material as the previous scaffold used in Case 3, also has been re-transplanted due to material fatigue.

6. No data is presented regarding the *"heavy", "significant", "chronic"* or *"necrotic" "inflammation"* or *"granulation tissue"* in the anastomotic regions, which repeatedly have been verified through histological analysis and bronchoscopies in Case 1 and 3.

7. No data is presented regarding the need for long-term hospitalization due to transplant complications. Case 3 is still hospitalized in the ICU over 2 years after the 1st transplantation. The patient is totally dependent on bronchoscopy every 4th hour, around the clock and daily aspiration of pus from the operative chest- and esophagus rest-cavities by 2 permanent chest drains through a persistent opening on the right chest wall.

8. No data is presented regarding the numbers of required post-transplant surgical interventions (requiring general anesthesia). In Case 3, 82 interventions have been performed between Jul 24, 2012 and submission of the article on Aug 4, 2013.
   (List of surgical procedures performed on Case 3 between Jul 24, 2012 and Aug 4, 2013, Appendix 35).

All of the above mentioned findings were clinically established, well known and registered in the Karolinska University Hospital medical records before the article was submitted on Aug 4, 2013, that is:

- 2 years and 2 months ***after*** Case 1 was transplanted on Jun 9, 2011.
- 1 year and 10 months ***after*** Case 2 was transplanted on Nov 17, 2011.
- 1 year ***after*** Case 3 was transplanted the 1st time on Aug 8, 2012 and 4 weeks ***after*** Case 3 was re-transplanted on Jul 9, 2013.

The tracheal re-transplantation in Case 3 (Jul 9, 2013) 11 months after the 1st transplantation (Aug 8, 2012) which implied 1 year of ICU-stay for the patient (still ongoing more than 2 years later) was performed due to a chronic infection and an highly insecure airway with multiple complications and requiring numerous interventions. Re-transplantation was performed by Prof Macchiarini nearly 4 weeks ***before*** submission of article on Aug 4, 2013.

This unique event of exchanging a synthetic trachea graft for a new synthetic tracheal scaffold after nearly 1 year of ICU care for highly complex airway pathology must have been fresh in his mind when preparing the manuscript. The reasons to omit this crucial and complex intervention, when at the same time admitting that Case 1 was in need for stenting, can only be speculated upon.
(Jul 9, 2013, Operation report of the tracheal re-implantation performed by Prof Macchiarini, Appendix 45).

In summary, this article demonstrates systematic presentation of inconsistent clinical, bronchoscopic and histological findings together with omission of known and registered data of crucial importance.

Article 6

Biomechanical and biocompatibility characteristics of electrospun polymeric tracheal scaffolds.

Ajalloueian F, Lim ML, Lemon G, Haag JC, Gustafsson Y, Sjöqvist S, Beltrán-Rodríguez A, Del Gaudio C, Baiguera S, Bianco A, Jungebluth P, Macchiarini P . *Biomaterials.* 2014 Jul;35(20):5307-15. Epub 2014 Apr 3.

(Appendix 13).Missing

Submitted Jan 13, 2014, published online Apr 3, 2014.

Major Inconsistencies:

1. Inquires have been unable to identify any application for synthetic tracheal transplantation filed at the Regional Ethical Review Board.
2. Unapproved informed consent form signed 17 days after the transplantation.
3. Omission of biopsy findings.
4. Omission of bronchoscopic findings.
5. Omission of clinical status and outcome.

Omission of Clinical Information:

In the Introduction section on the first page (p. 5307) the authors' states: ***"In 2011, the first synthetic-based tracheal scaffold, seeded with patient's autologous stem cells was transplanted in a clinical setting[1] [4].*** *This Y-shaped scaffold was manufactured from the preoperative chest CT and three-dimensional images of the patient trachea using a nanocomposite polymer (POSS-PCU; polyhedral oligomeric silsesquioxane [POSS] covalently bonded to poly-[carbonate-urea] urethane [PCU])[5]. U shaped rings of POSS-PCU were prepared through casting methodologies and were placed around a Y-shaped glass mandrel. Then, the whole construct was placed in a POSS-PCU solution, followed by a coagulation procedure which resulted in a porous scaffold [4]. However, due to the stiffness of the scaffold,* ***an abnormal granulation tissue formation developed within the post-operative course. Moreover, it led to chronic fistula at the distal anastomotic sites of the left main bronchus, which required endoscopic interventions."***

[1]Refers to Case 1 who was transplanted June 9, 2011. The patient died at Karolinska Jan 30, 2014.

General Comments:

1. The present article demonstrates the most severe form of omission of the vast clinical, bronchoscopic and histological pathological findings found registered in the Karolinska University Hospital medical records. The author chooses to present the clinical outcome in just 2 short sentences, thereby consciously omitting the major part of the continued and complex airway deterioration which lasted for over 2 years exposing the patient to several airway and gastro-intestinal interventions. After more than 1½-2 years with frequent re-admissions and a low quality of life, the patient succumbed to complications after nearly 6 months of hospitalization.

   The article was submitted on Jan 13, 2014, 4 weeks after the complicated esophageal transposition with a subcutaneous colon (large intestine) bypass from the neck to the abdomen (Dec 10, 2013) to treat a large tracheo-bronchial fistulation. The per-operative strategy had to be modified as it was deemed too risky to strip out the esophagus as the synthetic tracheal scaffold had detached at all anastomoses and would otherwise risk being completely dislocated.

   The Operation report (esophagus transposition with a subcutaneous colon bypass) Dec 10, 2013, describes:
   Section; Pre-operative evaluation:
   9th line: *"but has now developed a tracheo-bronchial fistulation and signs of that the tracheal graft has detached both proximally and distally.*
   Section; Progress of Operation:
   2nd line: ***"that from 25cm level and the whole way down to carina has the patients composite graft eroded into the forward wall of the esophagus. There is a large air leakage, especially distally."***
   6th line: ***"regarding this picture we consider that it would be to jeopardous to strip out the esophagus according to plan as the composite graft probably would dislocate completely.*** *Decide together with professor J. and professor L. to* ***install two new esophageal stents*** *in an attempt to cover the fistulation later to staple off the esophagus and leave it in situ and thereafter doing a bypass."*
   (Dec 10, 2013, Operation report, Appendix 48).

2. **More or less continuously hospitalized with a low quality of life since the beginning of June 2013, nearly 8 months *before* submission of the article (Jan 13, 2014**) the patients situation was described in a letter from the Department of Cardiothoracic Surgery, Reykjavik, Island to the Karolinska University Hospital dated Aug 3, 2013:

   "For over a year[1] XX[2] has suffered bothering respiratory symptoms that have only got worse*. It started with* ***recurrent hemoptysis*** *that required admission to our hospital. It was thought that these bleedings were related to granulation tissue at the anastomotic sites but potentially also from the stents. After intermittent treatment with Cycklocapron and bronchoscopic controls in Stockholm these problems have got better.* ***Instead, recurrent infections, mainly in XX right lung, have been the main concern for the last 8 months[3]****.* ***In December 2012[4] XX was diagnosed with a rather large abscess*** *in XX right lower lobe that gradually responded to iv. antibiotics.* ***Since then XX has been admitted multiple times to our hospital for copious blood tinged sputum and pneumonia-like symptoms.*** *Streptomonas mult bacteria have been grown from his trachea, but have been resistant to treatment."…*

   "Since early June[5] XX has been more in our hospital than at home. He is not septic but his problems with copious sputum and hemoptysis are worrisome and reduce his quality of life significantly."…

"With multiple investigations we have shown that XX lung is non-functioning. This is due to an early postoperative thrombus of a Vascutec graft to the right pulm artery and multiple distal embolies to the right lung. His right lung therefore seems to contribute minimally to his respiration."

[1]Approx. summer 2012 which means at least 8 months before submission of the article on Mar 18, 2013.
[2]Refers to Case 1 who was transplanted on June 9, 2011. The patient died at Karolinska on Jan 30, 2014.
[3] "last 8 months" was approx. 3 months before submission of the article on Mar 18, 2013.
[4] "December 2012" is 2-3 months before submission of the article on Mar 18, 2013.
[5]June 2013.
(Aug 3, 2013, Letter from the Department of Cardiothoracic Surgery, Reykjavik, Island Appendix 42).

3. **The patient was finally and terminally hospitalized on Sep 2013** (4½ months *before* submission of the article on Jan 13, 2014) until his death 5 months later on Jan 30, 2014 (17 days *after* submission of the article). Accordingly, the patient had been dead for over 2 months when the article was published on Apr 3, 2014.

4. The patient was continuously deteriorating in his respiration *before* and *after* submission of the article. He died 17 days *after* submission of the article due to a refractory respiratory insufficiency. This was a result of a nearly completely detached transplant, which was already visualized by the thoracic CT scan performed on Oct 22, 2013 (13 weeks ***before*** submission of the article on Jan 13, 2014).

   The CT report Oct 22, 2013, describes:
   3rd line: *"It seems to be* ***2 new established fistulations where 1) one communicates between the ventral esophagus and the distal dorsal part of the left transplant leg, and 2) the other is localized 1 cm cranially above and connects esophagus with the mediastinum and excides there in a circumferential and extended air-gap which surround the transplant at the carina level****. Except these pathological connections there are (as earlier) air filled fistulations which exits from the patient's own left main bronchus and communicates with irregular and extended air filled gaps in the mediastinum.* ***The contrast which has been ingested orally flows into both legs of the transplant, into the mediastinal air gaps as well as out into from bronchial tree cut off bronchuses in the right upper lobe and down in the known rest cavity in the right under lobe."***
   19th line: ***"Fistulations between the esophagus, the airways and the mediastinum. Progress of air filled gaps outside the tracheal transplant which probably now to the main part seems to be detached. Bronchiolitis and stagnation of secretion in the peripheral left lung."***
   (Oct 22, 2013, CT report thorax/esophagus, Appendix 49).

5. **Bronchoscopy on Sep 18, 2013 (4 months *before* submission of the article on Jan 13, 2014).**
   The report describes:
   5th line: *"Left side: the distal transplant ending lies almost in the inferior wall of the left main bronchus, there are some granulation tissue but the air can pass into the under lobe."*
   19th line: ***"Inspecting outside the stent, the sutures are there just sitting in the transplant without connection[1] to the native tissue."***
   [1]Implies a fully detached right anastomosis.

(Sep 18, 2013, Bronchoscopy report, Appendix 50).
(Sep 18, 2013, Bronchoscopy Film 12 on the USB device).

6. **Endoscopic insertion of 2 stents into the esophagus on Oct 30, 2013 (10 weeks *before* submission of the article on Jan 13, 2014)** as preparation before the planned esophageal surgery.
   The Operation report describes:
   1st line: *"Going down with the gastroscope indentifying the fistulation at 30 centimeters length from the teeth row, one can see the stent in the left main bronchus from the esophagus, the fistulation measures ca. 20% of the esophageal circumference. "*
   16th line: *"Thus there has been two esophagus-stents inserted against the fistulation."*
   (Oct 30, 2013, Operation report, Appendix 51).

7. **Bronchoscopy on Dec 17, 2013 (1 month before submission of the article on Jan 13, 2014).**
   The report describes:
   5th line: *at the proximal anastomosis "there is* ***a gap out to the mediastinum, covers maybe 20% of the rest of the anastomotic structures…"***
   8th line*: "when I move down into the graft there is an infection which moderately bubbles up from the right lung, on the* ***left side there is a stent located****, there is also* ***a stent located on the right side*** *down into the lung, it is a rather tight way distally down on the right side in the right bronchus. On the left side the stents are well positioned and runs into the distal part of the left main bronchus. There is pus in the lower lobe bronchus which bubbles up…"*
   16th line: *"There is indication of a* ***small leakage ca. 5 mm from the distal stent ending just prior to the plastic…"***
   23rd line: ***"…down into the grafts upper border, there is a rather large fistulation out over its edge out into the mediastinum…"***
   (Dec 17, 2013, Bronchoscopy report, Appendix 52).

   Comments:
   Together with the findings from the previous CT scan (Oct 22, 2013), the endoscopic intervention (Oct 30, 2013), the peri-operative observations (Dec 10, 2013) and the previous bronchoscopies with multiple stent interventions demonstrates more or less a catastrophic situation, which is briefly referred to as "***led to chronic fistula at the distal anastomotic sites of the left main bronchus, which required endoscopic interventions***. This is a gross example of omission of important clinical findings in a dying patient.

8. **The patient died on Jan 30, 2014,** 17 days *after* submission of the article on Jan 13, 2014 but 9 weeks *before* publication on Apr 3, 2014.
   Autopsy report from Feb 3, 2014, confirms a nearly full detachment of the transplant.

   In the Autopsy report under the section <u>*Respiratory organs*</u> the following is stated:
   5th line: *"Ca. 7 cm in the lower part of the throat is a eosophagobronchial fistulation. In the tracheal region there is a Y-shaped transplant detected with a thin and whitish component on its surface.* ***Around the transplant there is purulent fluid and necrotic tissue. Focally there are no native structures of the trachea and esophagus to be seen. The anastomotic endings towards the right and left bronchuses are disconnected and of the proximal anastomosis has 90% disconnected with sparsely adherent-like material between the graft and the distal end of the trachea."***
   (Feb 3, 2014, Autopsy report, Appendix 53).

Comments:
As suspected and verified several months before, no macroscopic sign of epithelialization or patent anastomoses, a synthetic scaffold nearly completely disconnected from its surroundings of necrotic tissue, cannot be described as anything else than catastrophic findings.

9. There doesn't seem to have been any application filed at the Regional Ethical Review Board before he was transplanted (Jun 9, 2011), which if confirmed, may be a severe violation against:

    a. Swedish Laws and Regulations on Ethical Vetting of Research Involving Humans (Ethical Review Act, Svensk Författningssamling 2003:460, Statute Jan 1, 2004, Amendment 2007/08:44, Statute Jun 1, 2008).
    b. Swedish Medical Devices and Pharmaceuticals Acts (Medical Product Agency, Läkemedelsverket).
    c. The Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects.