Begeleidingsformulier aanvraag dierproef DEC- UM	DECNR: 2011-066
Versie 2006 **Herziene versie**	Ontvangen: 26-05-2011

DEC datum goedkeuring#	Type aanvraag [2]	VROM/GGONR[3]	LNV/CBDNR[4]
26-05-2011	Nieuw		

Hoofdproject	CARIM
Deelproject	2

Financieel beheerder		Budgetnummer	3098.2299B

Titel van het onderzoek:
Characterization of Complex Fractionated Atrial Electrograms in Different Atrial Substrates

startdatum 01 June 2011 einddatum [9] 31 May 2014 *Duur van de proef [10]:* *10 weeks*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegdheid[5]	Cap. groep /afdeling
1. Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. overige uitvoerenden				Art.12 Art. 9 Art. 9	
5. PI				Art.9	

Diergroep	1	2	3	4				
ctrl/exp/sham	CTL	RAp	RVp	RAp+RVp				
Diersoort	42	42	42	42				
Stam	-	-	-	-				
Construct / mutatie ?	No	No	No	No				
Herkomst (leverancier) *	01	01	01	01				
Aantal	18	18	27	27				
Geslacht	M/F	M/F	M/F	M/F				
Dieren immuuncompetent ?	Yes	Yes	Yes	Yes				
Leeftijd/gewicht	40-60kg	40-60kg	40-60kg	40-60kg				
Doel van de proef *	31	31	31	31				
Belang van de proef *	01	01	01	01				
Toxicologisch onderzoek *	01	01	01	01				
Bijzondere technieken *	01	01	01	01				
Anesthesie *	04	04	04	04				
Pijnbestrijding *	04	04	04	04				
Mate ongerief *	03	03	05	05				
Toestand dier einde exp*	01	01	01	01				

* VHI coderingen zie bijlage

Verantwoording

Aanvraag dierproef DEC-UM

Titel: **Characterization of Complex Fractionated Atrial Electrograms in Different Atrial Substrates**

1. Doel van de proef.

Atrial fibrillation (AF) confers significant morbidity and mortality in patients and is set to become an epidemic. The cornerstone of catheter ablation procedures for AF involves isolation of pulmonary veins.[1] More recently, targeting of complex fractionated atrial electrograms (CFAEs) has been shown to improve success rates of catheter ablation in persistent AF patients.[2-3] However, our current understanding regarding the mechanisms underlying CFAEs remains limited.

This experiment aims to improve our understanding on the:
1) Effect of atrial substrate/remodeling on CFAEs development or types;
2) Electrophysiological & pathological characteristics of different CFAEs types;
3) Difference between 'critical' and 'by-standers' CFAES sites.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The increasing prevalence of this arrhythmia with overwhelming burden on healthcare systems worldwide demands our careful attention. This work will provide important scientific relevance to current clinical practice in targeting CFAEs during catheter based ablation procedures for AF. The ability to delineate 'critical' from 'by-standers' CFAEs sites will facilitate more targeted therapies, reduce procedural times and related complications.

3. Alternatieven

Humans cannot be used for this work as correlation between electrical properties and histology at CFAEs sites is not possible clinically. The swine model has been used extensively in basic arrhythmia research to provide an equivalent sized atrium with similar cardiovascular physiology as compared to humans'. Rapid pacing models have been used in this model to derive suitably remodeled atria from atrial, ventricular or combination pacing. The complexity of the AF substrate also renders computer or cellular modeling inferior and unsuitable.

4. Ethische afweging

The morbidity (including stroke and its related complications; symptoms such as palpitations, shortness of breath, chest pains or dizziness; requirement of toxic anti-arrhythmic medications or complex invasive ablation procedures; and recurrent hospitalizations) and mortality associated with AF is highly significant. Its burden on the healthcare system is likely to be in order of 1 billion Euros per year (extrapolated from Australian data, whereby the prevalence of AF is similar to The Netherlands – ~240,000 patients). At present, the reported success rates for catheter ablation procedures in persistent AF patients have remained stagnant. The know-how of where to ablate effectively beyond the pulmonary veins in the individual patient is simply missing due to our incomplete understanding of the disease mechanisms. This work will help to refine such ablation procedures by directing ablation to critical sites. In addition, the pharmacological component of this work will provide additional understanding of CFAEs useful, for future development of novel agents.

Wetenschap

5. Wetenschappelijke onderbouwing

Catheter ablation strategy of pulmonary vein isolation has been proven to be highly successful in patients with paroxysmal AF but remains inadequate in patients with more persistent form of this arrhythmia as a standalone technique.[4-5] The primary difference in these patient groups is the complexity of the underlying atrial substrate: persistent AF patients have more atrial structural remodeling due to concomitant cardiovascular or predisposing conditions than paroxysmal AF patients. These may include heart failure, hypertension, valvular disorders, obesity or sleep apnea. Although targeting of complex fractionated atrial electrograms (CFAEs) in addition to pulmonary vein isolation has been shown to improve our success in persistent AF patients, the results remain highly variable.[2,3,6]

Improved characterization of CFAEs is therefore important to help delineate critical CFAEs ablation targets from passive 'by-standers'. Indeed, CFAEs may represent zones harboring active AF drivers/rotors that maintain the arrhythmia or simply passive by-standers sites due to pivoting waves, wavefront collisions or conduction slowing.[7-9] Not surprisingly, one report showed that CFAEs are critical sites in only <10% of cases examined with the remaining accounted for by far-field signals or disorganized fibrillation activity.[10] Invariably, conduction block due to underlying anatomical changes has been highlighted to contribute to AF initiation but not its maintenance.[11] Ongoing multi-center prospective clinical trials are being conducted to examine different endocardial mapping techniques to guide ablation.[12] To date, there are only a handful of studies examining the characteristics of CFAEs even though they are frequently targeted in catheter ablation procedures. In addition, there remains a lack of correlation between endocardial CFAEs mapping with high density epicardial mapping or histology. We hypothesize that:

a) Electrical or structural remodeled atria will demonstrate different types of CFAEs;
b) 'Critical' & 'By-stander' CFAEs sites have different electro-pathological properties;
c) Pharmacological agents may differentiate 'Critical' from 'By-stander' CFAEs.

This project will involve high density epicardial mapping of AF in conjunction with real-time endocardial electroanatomical mapping using established clinical 3-D system. Established porcine models of electrically remodeled atria (rapid atrial pacing - RAp), structurally remodeled atria (rapid ventricular pacing - RVp) and a combination of both (RAp+RVp) will be utilized [13-15]. CFAEs sites will be studied in detail to correlate with underlying histological changes. Changes in CFAEs due to pharmacological agents - class I and III anti-arrhythmic drugs (AAD) and acetylcholine or adenosine; and conventional catheter based radiofrequency ablation will also be investigated. In summary, we aim to further characterize CFAEs in different atrial substrates using high density epicardial mapping and pathological correlate.

6. Wetenschappelijke beoordeling
The PI (mentioned on the front page) has read and approved this DEC proposal.

Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming
Pigs have been used widely in cardiovascular research. Specifically, other investigators have used this species successfully for the various rapid pacing models desired in this study.[13-15] The pigs have similar cardiovascular physiology and anatomy to humans. In addition, they offer better access to the posterior wall of the left atrium – a critical area in AF research, as compared to the commonly used goats. This will allow more extensive mapping of the posterior wall, required in this study. At the end of the experiments, all animals will be sacrificed and their heart tissues harvested for further histo-pathological analysis.

7b. Sexe
Both male and female pigs will be used in this experiment.

7c. Aantallen
A total of 90 animals are required for this research protocol. This estimation is based on the following calculation: The most variable electrophysiological parameter of our experiment: **atrial conduction**, has a standard deviation of about 20% (Estimates from previous works of Jens Eckstein and Dominik Linz - DEC 2006-063) We estimate a likely effect of 30%. Given a power of 80%, α=0.05, we need, according to the formula of Sachs, 6.98 animals per subgroup. A 20% dropout rate in all groups due to pacemaker problems, lead dislodgement, problems and possible health problems due to heart failure is anticipated. N= 6.98/0.8 = 8.72 → 9 animals per subgroup. In total 10 subgroups will be studied in this project. Total: 10 x 9 = 90 animals.

Dierproef

8. Experiment

We plan to have 4 main groups of animals to facilitate study of CFAEs in different atrial substrates:

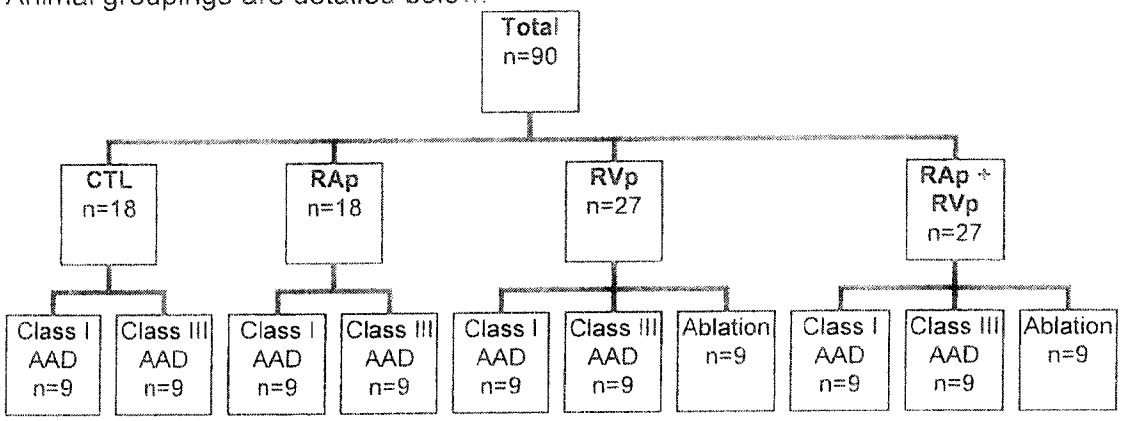
1) Sham-operated – CTL (n=18)
2) Rapid atrial paced – RAp (n=18)
3) Rapid ventricular paced – RVp (n=27)
4) Rapid atrial + ventricular paced - RAp+RVp (n=27)

The CTL group will have pacing leads implanted in the right atrium and right ventricle. The leads will be attached to a pacemaker with the device turned off (i.e. no pacing). The remaining groups will have the same atrial and ventricular leads implanted but will be paced according to their grouping. RAp animals will be paced in the atrium at 600bpm for 4 weeks to facilitate primarily atrial electrical remodeling. RVp animals will be paced in the ventricles at 200-240bpm for up to 4 weeks to achieve moderate degree of heart failure. RAp+RVp group will be paced simultaneously in the atrium and ventricle at 200-240bpm for up to 4 weeks to create a composite substrate of electrical and structural remodeling. To avoid rapid conduction from one chamber to another at all times, the AV node will be ablated in all animals at the time of pacemaker instrumentation.

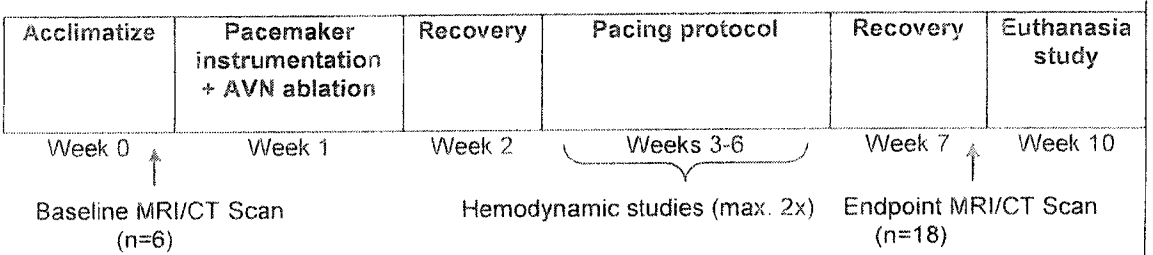
We will allow one week of recovery following initial instrumentation before commencement of the respective pacing protocol. In addition, a further week of recovery will be allowed for all animals following the conclusion of their respective pacing protocol before euthanasia electrophysiological evaluation. All animals will be closely monitored especially during the pacing period. Signs of heart failure such as impaired breathing, significant weight gain, peripheral edema, increased lethargy and reduced food intake will be actively recorded in the clinical record sheet. Additional hemodynamic measurements will be performed (maximum of 2) during the pacing period to better follow and document the progress of heart failure. Echocardiography will be performed as required to follow cardiac function. The pacing rate will be adjusted in the individual animal to achieve the desired degree of heart failure but this will be dictated by the clinical status of the animal with a primary goal of preventing undue distress due to heart failure. Diuretics therapy will be instigated for symptom relief whenever this becomes necessary.

The sacrifice experiment will involve detailed endocardial mapping in conjunction with a clinically utilized 3-D mapping system. In addition, high density epicardial plaques will be sited at key atrial locations for simultaneous recording. The heart will be removed at the end for detailed pathological analysis. Animals will be divided into the following subgroups: Pharmacological – Acetylcholine or adenosine with either Class I or Class III anti-arrhythmic drugs (AADs); Ablation – CFAEs ablation will be performed at highly fractionated areas. The above protocols allow for delineation of the electrophysiological basis and pathological correlation of CFAEs. The effect of ablation on CFAEs can also be examined in detail.

Animal groupings are detailed below:



Six randomly selected CTL animals will receive cardiac MRI/CT scans at study baseline prior to instrumentation and six in each of the following groups will be scanned prior to the euthanasia study: RAp, RVp and RAp+RVp. This will facilitate determination of in-vivo cardiac chambers size and function, merging of anatomy to endocardial maps obtained from the 3-D mapping system. The graph below shows the sequential intervention on a time-scale:



9. Experimentele condities

9a. Anesthesie

From 4pm day prior to anesthesia, pellet food will be withheld. Drinking water will be available ad libitum. For pacemaker implantations/AV nodal ablation, hemodynamic measurements and terminal studies:

- Pre-medication Zoletil (5-8mg/kg) i.m.
- Induction with thiopenthalnatrium (10-20mg/kg) i.v.
- General anesthesia. Ventilation with Air/O_2 (2:1). Analgesia and sedation will include Sufentanyl (6-12 µg/kg/h), Midazolam (0.5- 0.8mg/kg/h) & Propofol (2.5-10mg/kg/h).
- Replacement of volume loss with Ringer Lactate 10ml/kg/h i.v.

As the terminal studies will involve endocardial and epicardial mapping via open chest setup, a muscle relaxant will be used if required to facilitate ventilation:

- Pancuronium (0.3 mg/kg/h). Depth of anesthesia will be monitored closely using ECG and blood pressure.

9b. Pijnbestrijding

Pain will be evaluated by clinical factors (heart rate, breathing rate and pattern, behavior) and will be noted in the "welzijns dagboek".

- Analgesia - Rimadyl 4 mg/kg s.c. will be given for up to three days.
- Prophylactic antibiotics - 1g Ampicillin i.v. will be given peri-operatively and 1g i.m. postoperatively to cover pacemaker instrumentation.

9c. Euthanasie en Humane eindpunten

Terminal experiments will take place under general anesthesia as the SOP. The heart will be removed for further examination after completion of study protocol/ electrophysiological determinations.

Humane endpoints for this project will include the following:

- Recurrent infection of implantation site after antibiotic treatment;
- More than 1 pacing lead dislodgement;
- Significant weight gain/loss (>15% of expected weight per growth chart);
- Severe symptomatic heart failure; LV ejection fraction <15%
- Severe symptomatic heart failure; labored breathing,
- Severe symptomatic heart failure; respiratory rate > 40 breaths/minute
- Severe symptomatic heart failure; Edema

Zorg

10a. Ongerief

Control (CTL, n=18)

Procedure	Duration	Frequency	Discomfort
PM implantation + AV node ablation	1-2 hours	1x	03
MRI scan	1-2 hours	1x	02
Sacrifice experiment	12 hours	1x	02
Total			03

Electrical Remodeling (RAp, n=18)

Procedure	Duration	Frequency	Discomfort
PM implantation + AV node ablation	1-2 hours	1x	03
MRI scan	1-2 hours	1x	02
Development of AF	4 weeks	Ongoing	02
Sacrifice experiment	12 hours	1x	02
Total			*03*

Structural Remodeling (RVp, n=27)

Procedure	Duration	Frequency	Discomfort
PM implantation + AV node ablation	1-2 hours	1x	03
MRI scan	1-2 hours	1x	02
Development of heart failure	4 weeks	Ongoing	05
Hemodynamic measurement	1-2 hours	2x	03
Sacrifice experiment	12 hours	1x	02
Total			*05*

Combined Electrical and Structural Remodeling (RAp+RVp, n=27)

Procedure	Duration	Frequency	Discomfort
PM implantation + AV node ablation	1-2 hours	1x	03
MRI scan	1-2 hours	1x	02
Combined AF and heart failure	4 weeks	Ongoing	05
Hemodynamic measurement	1-2 hours	2x	03
Sacrifice experiment	12 hours	1x	02
Total			*05*

10b. Welzijnsevaluatie

The degree of distress will be comparable to previously approved protocols – DEC 2006-063, 2007-111 in terms of pacemaker instrumentations. Clinical condition of the animals will be scored daily by an evaluation sheet.

11. Verzorging en huisvesting

Pigs will be housed in CPV stables (inside and outside) in group housing when possible. Care and housing will be provided by CPV personnel. The experiments will be performed in our large animal theatre on In case of emergencies, VO and VVO can be contacted.

12. Deskundigheid

Our team consists of two skilled bio-technicians who have relevant experience and had undergone training in large animal surgery and anesthesia. They will be supported by the responsible researchers. All members of our team have art. 9 and/or 12 qualifications

13. Standard Operation Procedures (SOP)

General anesthesia

Pre-medication with Zoletil (5-8mg/kg) i.m.
The neck will be shaved and cleaned with alcohol.
The pig will be placed on the operating table.
Thiopenthalnatrium (10-20mg/kg) will be injected in an ear vein.
An endotracheal tube is placed in the trachea.
Ventilation is set at physiological settings, with expired CO_2 (4.0-4.5 %), O_2 saturation (90-100%) and ventilation pressure (pressure between 15 and 20 cm H_2O) as markers.
The hind leg will be shaved and cleaned with alcohol.
An intravenous catheter is placed in the vena cephalica.
Analgesia and sedation are provided by sufentanyl (6 µg/kg/h), Dormicum (0.8mg/kg/h) and propofol (10mg/kg/h). Pancuronium (0,3mg/kg/h) will only be given during long lasting open chest surgery.
Depth of anesthesia will be monitored by checking reflexes to sound and pain and ECG. In case of muscle relaxant blood pressure will be monitored closely.

Pacemaker implantations

After general anesthesia is induced, the operative field is shaved. Loose fur is removed by vacuum cleaner and the skin is washed with aseptic soap. The skin will then be disinfected with *jodium tinctuur 2%*. The jugular vein will be prepared. Then pacemaker leads (screw in, Medtronic) will be placed in the right atrial appendage and/or in the right ventricular apex under fluoroscopic control. The quality of the signal is judged and an injury current will be confirmation that the lead(s) is/are well attached to the myocardium. Pacing threshold is determined by an external pacemaker, pacing threshold is sufficient when it is 1.5 mA or lower. The vein will be closed and the lead(s) fixed with R knots. The leads will be externalized at the neck of the animal. The different wound layers will be closed with resorbable Polysorb 2-0

Ablation – AV node

After induction of general anesthesia, the operative field is shaved. Loose fur is removed by vacuum cleaner and the skin is washed with aseptic soap. The skin will then be disinfected with *jodium tinctuur 2%*. The femoral vein will be punctured with a Cook needle and venous sheath inserted for access to the atria. For AV nodal ablation, a good HIS signal will be located at the usual AV junctional spot. Radiofrequency ablation will be performed until AV block is evident. Following the procedure, the sheath will be removed and the puncture compressed to ensure hemostasis. If a cut-down is required to access the vein, different wound layers will be closed with resorbable Polysorb 2-0.

Hemodynamic studies

After general anesthesia is induced the operation field is shaved. Loose fur is removed by vacuum cleaner and the skin is washed with aseptic soap. The skin will then be disinfected with *jodium tinctuur 2%*. A catheter introduction sheet will be placed in the vena jugularis or vena cephalica and arteria carotis. Pressure will be measured in the right atrium, right and left ventricle, pulmonary artery and the aorta. Blood samples will be taken. After the measurements the vessels will be closed with a purse string suture. Different wound layers are closed with absorbable suture.

Cardiac MRI/CT scan

The animals will be transported to the . General anesthesia will be given prior to the scan. ECG, will be continuously monitored during the scanning process.

Terminal experiment

After general anesthesia is induced the operation field is shaved. Loose fur is removed by the vacuum cleaner and the skin is washed with aseptic soap. Catheter introduction sheets will be placed in the A. Carotis, V. Jugularis and femoral veins. Pressure catheters will be introduced to measure the arterial blood pressures. Two defibrillation paddles will be introduced to terminate AF during the protocol if necessary. One catheter will be positioned in the coronary sinus and two mapping/ablation catheters in the left atrium. A continuous infusion of heparin (5000 IU/h) is given to prevent clot formation at the endocardial catheters. The thoracic cavity will be approached trough a lateral incision. Pacing electrodes will be placed at the right and left atrium for electrophysiological measurements.

Custom built high density electrode plaques will be sutured to several left atrial sites for epicardial recording of AF electrograms. We will perform an electrophysiological mapping study of the atrium and ablation at the end of the protocol. The following pharmacological agents will be used during the mapping studies: Acetylcholine/adenosine with Class I or III AAD. After all the measurements have been done the heart will be excised and removed for in vitro experiments.

Relevante literatuur

[1] Haissaguerre M et al. N Engl J Med 1998; 339:659-66
[2] Nademanee K et al. J Am Coll Cardiol 2004; 43:2044-53
[3] Brooks AG et al. Heart Rhythm 2010; 7:835-46
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[6] Oral H et al. Circulation 2007; 115:2606-12
[7] Haissaguerre M et al. Circulation 2006; 113:616-25
[8] Kalifa J et al. Circulation 2006; 113:626-33
[9] Konings KT et al. Circulation 1997; 95:1231-41
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[11] Atienza F et al. J Am Coll Cardiol 2011; 57:1081-92
[12] Verma A et al. J Cardiovasc Electrophysiol 2011; In Press
[13] Bauer A et al. Cardiovasc Res 2004; 61:764-70
[14] Laurent G et al. Am J Physiol Heart Circ Physiol 2008; 294:H1206-15
[15] Chow E et al. Am J Physiol 1990; 258:H1603-5

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

, voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Aan:

Uw referentie:

Onze referentie

Maastricht, 03-05-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Characterization of Complex Fractionated Atrial Electrograms in Different Atrial Substrates*", is op de DEC vergadering van 29 april 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt bij punt 1 (doel van de proef, "in dit millennium" te verwijderen.
- Bij punt 7c verzoekt de DEC "the most variable" te benoemen.
- Bij punt 9b de tekst "twice a day postoperatively when appropriate" verwijderen.
- De DEC verzoekt de humane eindpunten meetbaar te definiëren.

Gelieve eventuele vragen te beantwoorden in een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.
Uw project staat bij de DEC geregistreerd onder nummer 2011-066, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC -UM

To: DEC-UM

From:

Date: 19 May 2011

Re: DEC 2011-066

Please find attached the amended experimental protocol following your recommendations. Specifically:

- De DEC verzoekt bij punt 1 (doel van de proef, "in dit millennium" te verwijderen.

  These words are now removed.

- Bij punt 7c verzoekt de DEC "the most variable" te benoemen.

  We have specified this to be atrial conduction.

- Bij punt 9b de tekst "twice a day postoperatively when appropriate" verwijderen.

  This has been altered.

- De DEC verzoekt de humane eindpunten meetbaar te definiëren.

  This section is now clearly defined.

Thank you for your kind attention.

Yours sincerely,