Anorectal Manometry May Reduce the Number of Rectal Suction Biopsy Procedures Needed to Diagnose Hirschsprung Disease
Meinds, Rob; Trzpis, Monika; Broens, Paulus

Published in:
Journal of Pediatric Gastroenterology and Nutrition

DOI:
10.1097/MPG.0000000000002000

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 24-02-2019
Anorectal Manometry May Reduce the Number of Rectal Suction Biopsy Procedures Needed to Diagnose Hirschsprung Disease

Rob J. Meinds, Monika Trzpis, and Paul M.A. Broens

ABSTRACT

Objectives: The aim of the study was to evaluate whether anorectal manometry (ARM), which is used to test the rectoanal inhibitory reflex (RAIR), is a safe alternative for reducing the number of invasive rectal suction biopsy (RSB) procedures needed to diagnose Hirschsprung disease (HD).

Methods: Between 2010 and 2017, we prospectively collected the ARM results of 105 patients suspected of having HD. Following the outcome, the patients either underwent additional tests to confirm HD or they were treated conservatively. Primary ARM-based diagnoses were compared with the definitive diagnoses based on the pathology reports and/or clinical follow-ups. Additionally, we analyzed whether modifications to our ARM protocol improved diagnostic accuracy.

Results: The sensitivity of ARM and RSB was comparable (97% vs 97%). The specificity of ARM, performed according to our initial protocol, was significantly lower than that of RSB. After we modified the protocol to confirm the definitive diagnosis of HD or to discard it. Unfortunately in the past, performing ARMs in extremely young patients can, however, be unreliable and 17% of the biopsies need to be repeated due to inconclusive test results (3). In addition, RSBs are invasive and carry the risk of complications.

Conclusions: ARM is a viable screening tool for HD and, provided it is performed properly, it can be used to exclude HD with absolute certainty. By contrast, an absent rectoanal inhibitory reflex on ARM should always be followed by an RSB to confirm the diagnosis of HD. Using ARM as the diagnostic of first choice could reduce the number of invasive biopsies.

Key Words: constipation, diagnosis, infants, newborns, screening

What Is New

- The current most commonly used diagnostic procedure for Hirschsprung disease is rectal suction biopsy, which has its limitations and carries a risk of complications.
- Anorectal manometry is a noninvasive tool with the potential to reduce the number of invasive biopsy procedures.

Constipation is common among infants and newborns (1). In rare cases constipation may be caused by Hirschsprung disease (HD), a congenital absence of ganglion cells, aganglionosis, of the enteric nervous system. Currently, rectal suction biopsy (RSB) is the most commonly used procedure for the diagnosis of HD (2). RSBs in extremely young patients can, however, be unreliable and 17% of the biopsies need to be repeated due to inconclusive test results (3). In addition, RSBs are invasive and carry the risk of complications (4). Taking into account these limitations, in combination with the high prevalence of constipation in the pediatric population, we propose the use of a less invasive tool to reduce the number of invasive RSBs.

Anorectal manometry (ARM) is just such a noninvasive tool that carries little or no risks. ARM consists of dilating a rectal balloon and measuring the response in anal sphincter pressure. In healthy individuals, rectal balloon stimulation is followed by a rectoanal contractile reflex (RACR) and a rectoanal inhibitory reflex (RAIR) (5). In patients with HD, however, the RAIR is absent (6–8). A RAIR found by using ARM thus obviates the need for RSB as HD has virtually been excluded. By contrast, not finding a RAIR may be an indication of HD or to discard it. Unfortunately in the past, performing ARMs in extremely young patients was disputed because of the difficulties encountered in doing so (2,9). As a consequence, only a small percentage of pediatric surgeons still uses ARM in the diagnosis of HD, while the majority opts for RSB as the diagnostic of first choice (10,11). This may not
be entirely justified, because the value of ARM as a useful screening tool for HD has been demonstrated repeatedly (9,12–16).

In recent years, modifications to our ARM protocol have helped us to increase its diagnostic accuracy. We hypothesize that with these improvements, ARM could be used to reduce the number of invasive biopsies needed to diagnose HD, and to serve as a complement to RSB in the diagnosis of other causes of constipation in infants and children. Our aim is therefore to evaluate whether ARM performed with our modified protocol is a viable and safe screening tool for HD.

METHODS

Study Design

Between 2010 and 2017, we prospectively collected data on 105 patients who were suspected of having HD and who underwent ARM at the Anorectal Physiology Laboratory of the University Medical Center Groningen. The inclusion criteria and indications for performing ARM were delayed meconium passage, distended abdomen, difficult spontaneous defecation, and/or signs of HD-associated enterocolitis. There were no exclusion criteria. A pediatric surgeon with many years of ARM experience analyzed and interpreted all the measurements blindly and independently from other clinical data. Based on the outcomes of the ARM tests and the patients’ clinical condition, patients either underwent additional testing, such as RSB or full-thickness biopsies, or they were treated conservatively with laxatives and rectal washouts. The age at which the biopsies were performed was corrected for the infants’ gestational age, whereby a minimal duration of 38 weeks was considered normal. The medical ethics committee of University Medical Center Groningen approved the study.

Anorectal Manometry Procedure

Measuring Equipment

We recorded and analyzed the data with solar gastrointestinal high-resolution manometry equipment (version 9.30; Laborie/Medical Measurement Systems, Enschede, the Netherlands). We used a Laborie (Unisensor) K12959 catheter with an outer diameter of 12F, circumferential pressure sensors taking a reading every 8 mm over a total length of 5.6 cm, and a microtip sensor within a small, nonlatex balloon attached to the tip of the catheter to inflate it and to register the pressure inside the balloon.

Anorectal Manometry Protocol

A few minutes before insertion the catheter was warmed-up in water at body temperature, after which a small amount of inert gel was applied to the balloon. The level to which the catheter was inserted depended on the age of the patient; preferably it was inserted until the last pressure sensor was visible at the edge of the anal canal. Once it was in place, the catheter was fixed to the patient’s buttocks with tape. After insertion a few minutes were allowed for the anal sphincter pressure to return to base value. At intervals of at least thirty seconds the rectoanal reflexes were measured by inflating the rectal balloon with increasing volumes of air that were rapidly injected and ejected after 1 second.

During the course of the study we modified the ARM protocol significantly by adjusting the maximum dilatation volumes of the rectal balloon. In the initial protocol, which was used from 2010 to 2014, the balloon was inflated with small steps of, for example 1, 2, or 3 mL at a time, until the dilatation volume was considered maximal for the age of the patient. In the modified protocol, which we used from 2014 to 2017, the balloon was inflated with steps of, for example, 1, 3, 5, or 8 mL, until we observed either the RACR or the RAIR. Even though our safety protocol mentioned that inflation should only be increased until resistance to inflating the balloon increased or the patient showed signs of discomfort, none of our patients reached this level.

Anorectal Manometry Interpretation

The RACR was defined as a rapid and temporary increase of anal sphincter pressure of at least 10 mm Hg directly following balloon dilatation. The anal pressure difference had to be significantly more pronounced with increasing rectal dilatation (Fig. 1B). A functioning RAIR was defined as a decrease in anal sphincter pressure of at least 20 mm Hg following balloon dilatation. As depicted in Figure 1A, the difference in anal pressure has to be significantly more pronounced with increasing rectal dilatation. We did not consider spontaneous relaxations of the anal sphincter, without direct preceding rectal dilatations, to be rectoanal inhibiting relaxations (Fig. 1C). We defined ARM as positive for HD if the RACR was absent, ARM was defined as inconclusive if there was no clear relaxation of the anal sphincter, that is, a threshold of 20 mm Hg relaxation was not reached, or if the morphology of the RAIR was abnormal. Lastly, ARM was defined as negative for HD if the RACR was present, with increasing relaxation of the anal canal with increasing rectal dilatations.

Rectal Suction Biopsy Procedure

The RSB procedure we used consists extracting specimens at 3 levels, that is, 3, 4.5, and 6 cm above the anal verge. Subsequently, the specimens were stained with hematoxylin and eosin, nicotinamide adenine dinucleotide enzyme histochemistry, and acetylcholinesterase histochemistry. We had to exclude 10 RSBs due to insufficient tissue for the diagnosis of HD. The remaining RSBs were classified as positive for HD, that is, no ganglion cells and/or increased nerve fiber proliferation, inconclusive for HD, that is, no ganglion cells and little or no nerve fiber proliferation, or negative for HD, that is, ganglion cells and little or no increased nerve fiber proliferation.

Data Analysis

We compared the outcomes of ARM with the final diagnoses based on the pathology reports and/or clinical follow-ups. On the basis of the final diagnoses, the ARM test results were classified as true positive, false positive, true negative, false negative, or inconclusive. Using these criteria, we determined diagnostic aspects such as sensitivity, specificity, positive predictive value, and negative predictive value for ARMs. The same was done for the RSBs. By doing so, we were able to compare the diagnostic aspects of ARMs and RSBs.

In order to analyze the outcomes of ARM at different ages, we divided the patients into 3 equally sized groups on the basis of their age percentiles: −14 to 65, 66 to 167, and 168 to 5532 days of age (corrected for gestational age).

We modified the ARM protocol on the basis of our experience. To test the effect of these modifications, we compared the diagnostic aspects of ARMs performed according to the initial protocol (n = 64) to those performed according to the modified protocol (n = 41).

Statistical analysis

Data were analyzed with IBM SPSS Statistics 23 for Windows (IBM Corporation, Armonk, NY). We reported continuous values as medians with range. Statistical tests were limited to
Pearson’s chi-squared test and the Mann-Whitney tests. Sensitivity and specificity values were defined as the proportion of positives and negatives correctly identified as such. Positive and negative predictive values were defined as the proportions of positive and negative results that were defined as true-positive and true-negative test results, respectively. Two-sided \( P \) values of less than 0.05 were considered statistically significant.

RESULTS

Patient Characteristics

A total of 105 patients suspected of having HD and who had undergone ARM were included. The majority of patients was male (63%, \( n = 66 \)). Patients’ median gestational age was 39 weeks (range 26–42 weeks). The first presenting symptom was a delayed meconium passage in 41% (\( n = 43 \)) of the patients, chronic constipation in 55% (\( n = 58 \)), sigmoid volvulus in 2% (\( n = 2 \)), and an intestinal perforation in 2% (\( n = 2 \)). Following the ARM, 1 or more RSBs were performed in 79% of the patients (\( n = 83 \)), while 15% (\( n = 16 \)) required a full-thickness biopsy to arrive at the final diagnosis (Fig. 2). The remaining 15 patients (14%) did not require a biopsy because at follow-up constipation had resolved completely or near-completely. Ultimately, we diagnosed 34% (\( n = 36 \)) of the patients with HD (Fig. 2).

Anorectal Manometry Outcomes

Patients’ age at the time of ARM ranged from –14 days (preterm birth, corrected for gestational age) to 5532 days, that is, 15 years, with a median age of 114 days. Over the entire study period, we found that the sensitivity of ARM was 97%, while its specificity was 42% (Table 1). A positive ARM result had a positive predictive value of 56% for eventual HD. Notably, a negative ARM result excluded HD with absolute certainty, the negative predictive value being 100%.

We found no significant differences in diagnostic accuracy of ARM among the 3 age groups investigated (Supplementary Table 1, Supplemental Digital Content, http://links.lww.com/MPG/B358). The sensitivity ranged from 94% to 100% (\( P = 0.526 \)) and the specificity ranged from 35% to 48% (\( P = 0.580 \)).
Rectal Suction Biopsy Outcomes

We analyzed a total of 83 RSBs performed in 77 patients. The median age at the time of RSB was 83 days, with a minimum of −14 days (preterm birth, corrected for gestational age) and a maximum of 1522 days, that is, 4 years. Overall, the sensitivity of the RSBs was 97% and specificity was 84% (Table 1). Moreover, RSBs had a positive predictive value of 97% and a negative predictive value of 100%.

Comparison of Anorectal Manometry and Rectal Suction Biopsy

Additionally, we compared the outcomes of the results of 105 ARMs with the results of 83 RSBs and found no significant difference in sensitivity between the 2 tests (Table 1). The specificity of RSBs, however, we found to be significantly higher than that of ARMs (84% vs 42%, \( P < 0.001 \)). Moreover, the positive predictive value of RSBs was significantly higher than that of ARMs (97% vs 56%, \( P < 0.001 \)). The negative predictive value of both tests was 100%.

One patient had a false-positive RSB result. While the biopsies taken at 4.5 and 6 cm from the anal verge showed normal nerve innervation, the biopsy taken at 3 cm showed complete absence of ganglion cells and a proliferation of AChE positive nerve fibers, compatible with short segment HD. The ARM performed in this patient, however, showed a functioning RAIR at a dilatation of 5 mL, contradicting the diagnosis of HD. Following a conservative treatment with rectal washouts and laxatives, constipation had completely disappeared at follow-up within 1 year.

TABLE 1. Comparison of anorectal manometry and rectal suction biopsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Outcome</th>
<th>Anorectal manometry (total group, ( n = 105 ))</th>
<th>Rectal suction biopsy (( n = 83 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD patients</td>
<td>True positive (n)</td>
<td>35/36 (97%(^a))</td>
<td>33/34 (97%(^a))</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>Inconclusive (n)</td>
<td>1/36 (3%)</td>
<td>1/34 (3%)</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>False negative (n)</td>
<td>0/36 (0%)</td>
<td>0/34 (0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>True negative (n)</td>
<td>29/69 (42(^b))</td>
<td>41/49 (84(^b))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-HD patients</td>
<td>Inconclusive (n)</td>
<td>13/69 (19%)</td>
<td>7/49 (14%)</td>
<td>0.516</td>
</tr>
<tr>
<td></td>
<td>False positive (n)</td>
<td>27/69 (39%)</td>
<td>1/49 (2%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Outcome</th>
<th>Anorectal manometry (modified protocol, ( n = 41 ))</th>
<th>Rectal suction biopsy (( n = 83 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD patients</td>
<td>True positive (n)</td>
<td>7/7 (100(^a))</td>
<td>33/34 (97(^a))</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>Inconclusive (n)</td>
<td>0/7 (0%)</td>
<td>1/34 (3%)</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>False negative (n)</td>
<td>0/7 (0%)</td>
<td>0/34 (0%)</td>
<td>0.260</td>
</tr>
<tr>
<td></td>
<td>True negative (n)</td>
<td>25/34 (74(^b))</td>
<td>41/49 (84(^b))</td>
<td>0.452</td>
</tr>
<tr>
<td>Non-HD patients</td>
<td>Inconclusive (n)</td>
<td>3/34 (9%)</td>
<td>7/49 (14%)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>False positive (n)</td>
<td>6/34 (18%)</td>
<td>1/49 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

HD = Hirschsprung disease.
\(^a\) Sensitivity.
\(^b\) Specificity.
Finally, following an RSB, 1 patient had rectal blood loss and required hospitalization and surgical hemostasis. No complications occurred during any of the ARM tests.

**Comparison of Anorectal Manometry Protocols**

Next, we compared the initial ARM protocol (n = 64) with the modified ARM protocol (n = 41) (Table 2). One of the main differences between the 2 protocols was that in the modified protocol the balloon was inflated until either a RACR or a RAIR was elicited. This method meant that the maximal volume used for dilatation was significantly higher in the modified protocol than in the initial protocol (median 9.5 vs 3 mL, P < 0.001). Using this modified protocol we induced a RACR in 78% of the patients, whereas in the initial protocol this was 13% (P < 0.001). Modification of the protocol also resulted in an increased prevalence of the RAIR (68% vs 17%, P < 0.001).

The use of higher dilatation volumes in the modified protocol drastically increased specificity in comparison to the initial protocol (74% vs 11%, P < 0.001, Table 2). The other diagnostic aspects, such as sensitivity, positive predictive value, and negative predictive value, were not significantly different between the 2 protocols (Table 2).

Lastly, we compared the outcomes of the modified ARM protocol (n = 41) with the results of RSBs (n = 83, Table 1). After modification, the specificity of the ARMs increased and was no longer significantly different from the RSBs (74% vs 84%, P = 0.260). Moreover, there were no significant differences in sensitivity between the modified ARM protocol and the RSBs (100% vs 97%, P = 0.646).

**DISCUSSION**

While ARM is widely accepted as a diagnostic tool in older children and adults suspected of having HD, its use in newborns is disputed because of the possibility of finding false negatives and high sensitivity, positive predictive value, and negative predictive value, which may have also resulted in a lower specificity and a reduced positive predictive value. Last, inability to elicit the RAIR could also be the result of a delay in the development of this reflex. The literature does not agree on this issue. One study showed that the RAIR does indeed develop after birth (17) while another study found that the RAIR is already present at birth, even in preterm-born infants (18). Further research is required to determine whether development of the RAIR in particular or development of the physiology of the anal canal in general, plays a role in constipation in newborns. The false-positive ARM results do, however, mean that at all times it remains necessary to perform an RSB in case no RAIR was found by ARM.

While RSB is currently the most commonly used tool for diagnosing HD (2), it may also have its shortcomings. First, RSBs have a significantly lower sensitivity in patients younger than 38 days in comparison to older patients (3). Moreover, RSBs also seem to carry the small risk of false HD diagnoses, as demonstrated by the false-positive RSB outcome in our current study that was later disputed by the ARM test result and clinical follow-up. Lastly, while a RSB is generally considered safe, the risk of complications remains due to its invasive nature (4). We too had 1 serious complication following RSB, which had to be treated surgically. Aside from this complication, we had to exclude of the biopsies because of insufficient material, after which the biopsies had to be repeated. These negative experiences with RSBs are corroborated by the reports of others (19,20). These limitations, we believe, can be overcome partially by utilizing the advantages of ARM—the main advantage being that it is non-invasive and therefore carries no risks of complications.

The primary limitation of our study was that not all patients underwent a biopsy to confirm or exclude the diagnosis of HD. In patients with HD, all the diagnoses were confirmed by RSBs and in the majority of cases the diagnosis was confirmed by postoperative pathological inspection of the intestinal resection. Not every patient in whom HD had been excluded, however, underwent an RSB procedure. As a consequence, ARM test results may have been wrongly labeled as true negative, thus overestimating diagnostic accuracy. We do believe, however, that if the diagnosis of HD had been missed at a young age, constipation would most likely have

<table>
<thead>
<tr>
<th>TABLE 2. Comparison of anorectal manometry protocols</th>
<th>Initial protocol n (%)</th>
<th>Modified protocol n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>64 (100)</td>
<td>41 (100)</td>
<td></td>
</tr>
<tr>
<td>Rectoanal reflex tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functioning RAIR</td>
<td>11/64 (17)</td>
<td>28/41 (68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RAIR stimulation threshold, mL. *</td>
<td>2 (1–15)</td>
<td>5 (1–15)</td>
<td>0.005</td>
</tr>
<tr>
<td>Functioning RACR</td>
<td>8/64 (13)</td>
<td>32/41 (78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RACR stimulation threshold, mL. *</td>
<td>4.5 (2–10)</td>
<td>4 (1–50)</td>
<td>0.805</td>
</tr>
<tr>
<td>Maximum balloon dilatation, mL.</td>
<td>3 (1–30)</td>
<td>9.5 (4–60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnostic accuracy measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>28/29 (97)</td>
<td>7/7 (100)</td>
<td>0.618</td>
</tr>
<tr>
<td>Specificity</td>
<td>4/35 (11)</td>
<td>25/34 (74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>28/49 (57)</td>
<td>7/13 (54)</td>
<td>0.831</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>4/4 (100)</td>
<td>25/25 (100)</td>
<td></td>
</tr>
</tbody>
</table>

RACR = rectoanal contractile reflex; RAIR = rectoanal inhibitory reflex.
*Median (range).
persisted and the diagnosis would have been reconsidered at a later age. Recently, we experienced such cases in 2 patients who were eventually diagnosed with HD at 18 and 14 years of age (21). Another limitation of this study may be the setting, namely a tertiary referral center with a relatively high prevalence of HD (up to 34% in this study). This limitation may have partially biased the predictive values we found, as these are affected by the disease prevalence (22).

Lessons Learned Using Anorectal Manometry to Exclude Hirschsprung Disease

As mentioned before, HD is excluded once ARM reveals that RAIR is activated upon rectal dilatation, which is a safe and noninvasive procedure. Because the rectal volumes differ between patients, it is, however, difficult to standardize the volumes required for successfully eliciting the RAIR. Lack of such standardization has raised doubts about the reliability of ARM, especially in terms of reproducibility. Unfortunately, many medical centers have therefore opted for RSB as the first-choice diagnostic (10,11), which seems to be technically easier to perform than ARM. If the benefit for the patient is taken into account, that is, the noninvasive nature of ARM, and the high specificity and sensitivity described before (2), and also in this study, we, however, think that ARM should be considered in every center’s HD workup protocol.

During the study we learned several valuable lessons that helped us improve the diagnostic accuracy of ARM. First, we found that it is important to accurately register the precise moment the balloon is inflated (Fig. 1C). Such accurate registration is necessary to determine whether a difference in sphincter pressure is caused by random relaxation or whether it occurred as result of a rectal dilatation. Second, we learned that in case of a functional RAIR, relaxation following balloon dilation should become deeper and longer when the balloon is increasingly inflated (Fig. 1A). Continuing to inflate the balloon, even after having found a functional RAIR, further reduces the risk of a false-negative test result. The last and most important lesson we learned is that it is important to keep on inflating the balloon until a RACR is elicited (Fig. 1B). One of the reasons for a false-positive test result is inadequate stimulation of the rectal wall because, in some constipated children and newborns, the rectal volume may be larger than is to be expected considering their age. In such patients, dilation of 5 mL will be insufficient to stimulate the rectal wall. In the majority of patients, a dilatation with at least 10 mL was necessary to elicit the RAIR.

CONCLUSIONS

ARM is a viable screening tool for excluding HD, provided the correct technical improvements are made. Importantly, a functioning RAIR found by ARM obviates the need for an RSB, because HD has virtually been excluded. By contrast, no functioning RAIR warrants an RSB to confirm or exclude the diagnosis of HD. By using ARM as the diagnostic of first choice the number of invasive biopsies, and therefore the risk of complications, can be reduced. This study can form the starting point toward a standardized method for the measuring and appraisal of anorectal reflexes in HD.

Acknowledgments: The authors thank Mrs O.J. Pras, Mrs T. de Groot, Ms S. Gerritsen, and Mrs B. Brongers-Posthuma of the Anorectal Physiology Laboratory for their substantial assistance in performing the measurements. In addition, we would like to thank T. van Wulfften Palthe, PhD, for correcting the English manuscript.

REFERENCES