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*Research Article*

# THE EFFECT OF *LACTOBACILLUS CASEI RHAMNOSUS* (LCR35) SUPPLEMENTATION ON THE ADHERENCE, TOLERANCE AND EFFICACY OF *HELICOBACTER PYLORI* ERADICATION THERAPY: AN OPEN-LABEL, OBSERVATIONAL, NON-INTERVENTIONAL, MULTICENTRE PILOT STUDY

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**ABSTRACT:** ***Approximately 30% of patients receiving Helicobacter pylori (HP) eradication therapy develop antibiotic-associated diarrhoea (AAD). Premature treatment discontinuation is an important cause of resistance to antibiotics and falling response rates. Austria is considered a high-resistance country with HP eradication rates in first-line sequential standard triple therapy (SST) rarely exceeding 70%. The probiotic Lactobacillus casei rhamnosus strain 35 (LCR35) is approved for treatment of AAD. This non-interventional study (NIS) evaluated the efficacy and safety of LCR35 in clinical practice in patients with clinically relevant and histologically confirmed HP infection receiving SST. Patient adherence, incidence of AAD and response to SST were documented. Out of 112 patients, 107 (95.5%) adhered to SST, 102 (91.1%) responded to SST, 7 (6.3%) showed adverse events, mainly gastrointestinal and 3 (2.8%) of them developed AAD. Despite inherent limitations of a NIS, these results suggest that LCR35 may reduce the AAD rate, increase patient adherence, and increase HP eradication rates.***

**KEY WORDS:** Antibiotic-associated diarrhoea**;** *Helicobacter pylori*; *Lactobacillus casei rhamnosus* LCR35

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## INTRODUCTION

*Helicobacter pylori* (HP) infections are a common and significant health problem worldwide. In Western Europe, the prevalence of HP infections ranges between 32% (Netherlands) to 84% (Portugal) in adults and 11% (Belgium) to 66% (Portugal) in children. In North America and Northern Europe, the prevalence of HP infections is estimated to be as high as one third of the population (Eusebi et al., 2014). HP eradication is indicated in patients with peptic ulcer disease (active or not, including complicated ulcer), MALT lymphoma, atrophic gastritis, post-gastric cancer resection, in patients who are first-degree relatives of gastric cancer patients, and upon patients' wishes (after full consultation with their physician) (Malfertheiner et al., 2002). In Austria, the standard sequential therapy (SST) is based on proton-pump inhibitors (PPI), amoxicillin and clarithromycin (or metronidazole) and levofloxacin, or quadruple non-bismuth sequential therapy (SEQ) in patients with known clarithromycin resistance (Högenauer et al., 2014; Malfertheiner et al.; 2017). Approximately 30% of patients are affected by undesirable side effects of antibiotic therapy (McFarland 1998; Barbut and Meynard, 2002) potentially impairing treatment outcome on an individual and global level, as affected patients have a strong tendency to discontinue their antibiotic therapy prematurely, which in turn may trigger the development of antibiotic-resistant HP strains (Dang et al., 2014). Growing resistance to standard antibiotic therapy is a rising concern. Eradication rates of 75% for STT comprising a PPI, clarithromycin and amoxicillin and 82% for non-bismuth quadruple SEQ, respectively, have been reported by the Cochrane Collaboration (Nyssen et al., 2016). This Cochrane systematic review reported a 1.72% yearly reduction in the efficacy of SEQ and a 0.9% yearly reduction for STT (Nyssen et al., 2016). There is increasing evidence that probiotics may be useful in supporting the efficacy of antibiotic-based HP eradications schemes (Zhang et al., 2015). A systematic

review of 45 randomized, controlled trials reported that the use of probiotics plus standard therapy was associated with an 11% increased eradication rate in the per-protocol analysis (RR = 1.11; 95%CI: 1.08-1.15; $P < 0.001$) and a 13% increased eradication rate in the intention-to-treat analysis (RR = 1.13; 95%CI: 1.10-1.16; $P < 0.001$), while the incidence of adverse events was reduced by approximately 15% (21.44% in the probiotics group versus 36.27% in the control group) (Zhang et al., 2015). Based on such findings, the Maastricht V/Florence Consensus Report (Malfertheiner et al., 2017) as well as the German S2k guidelines (Fischbach, Malfertheiner et al. 2016) recommend the use of probiotics to reduce antibiotic-associated diarrhoea (AAD) and increase eradication rates and adherence to treatment. However, a recent review of the literature showed that across all available experimental studies and randomized, controlled trials only few probiotic *Lactobacillus* strains displayed *in vitro* or *in vivo* efficacy in antagonizing HP infection or improving the eradication rate of the triple therapy in the clinical setting (Lievin-Le Moal and Servin, 2014). Special consideration should also be granted to the distinct properties and manufacturing as well as regulatory standards of medicinal-quality versus food-quality probiotics (Schulze et al., 2008).

**TABLE 1. HP eradication plan adopted by different investigators.** SST, sequential standard triple therapy consisting of PPI 2x1 units, amoxicillin 2x1 g, followed by PPI 2x1 units, levofloxacin 2x500 mg, metronidazole 2x500 mg; ST, standard triple therapy consisting of PPI 2x1 units, clarithromycin 2x500 mg, amoxicillin 2x1 g

| Type | Centre | Active agents and dose | Administration schedule | | N (%) in the plan |
|---|---|---|---|---|---|
| SST | Dr. Tonninger-Bahadori | LCR35 1.5 g powder | 1 - 0 - 0 | Day 1-5 | 55 (49.1%) |
| | | Amoxicillin 1 g | 1 - 0 - 1 | | |
| | | Omeprazol 40 mg | 1 - 0 - 1 | | |
| | | LCR35 1.5 g powder | 1 - 0 - 0 | Day 6-10 | |
| | | Levofloxacin 500 mg | 1 - 0 - 1 | | |
| | | Metronidazol 500 mg | 1 - 0 - 1 | | |
| | | Omeprazol 40 mg | 1 - 0 – 1 | | |
| ST | Dr. Tonninger-Bahadori, | LCR35 1.5 g powder | 1 – 0 – 0 | Day 1 - 14 | 2 (1.8%) |
| | penicillin allergy-schedule | Levofloxacin 500 mg | 1 – 0 – 1 | | |
| | | Clarithromycin 500 mg | 1 – 0 - 1 | | |
| | | Omeprazol 40 mg | 1 – 0 - 1 | | |
| SST | Dr. Uitz | LCR35 1.5 g powder | 1 -0 - 1 | Day 1 - 5 | 23 (20.5%) |
| | | Amoxicillin 1 g | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | | |
| | | LCR35 1.5 g powder | 1 -0 - 1 | Day 6-10 | |
| | | Levofloxacin 500 mg | 1 -0 - 1 | | |
| | | Metronidazol 500 mg | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | | |
| | | LCR35 1.5 g powder | 1 -0 - 1 | Day 11 - 20 | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | Day 11 - 38 | |
| ST | Dr. Uitz, | LCR35 1.5 g powder | 1 -0 - 1 | Day 1 - 7 | 8 (7.1%) |
| | alternative schedule | Clarithromycin 500 mg | 1 -0 - 1 | | |
| | | Amoxocillin 1 g | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | Day 8 - 28 | |
| SST | Dr. Bahadori | LCR35 1.5 g powder | 1 -0 - 1 | Day 1 - 5 | 24 (21.4%) |
| | | Amoxicillin 1 g | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | | |
| | | LCR35 1.5 g powder | 1 -0 -1 | Day 6-10 | |
| | | Levofloxacin 500 mg | 1 -0 - 1 | | |
| | | Metronidazol 500 mg | 1 -0 - 1 | | |
| | | Pantoprazol 40 mg | 1 -0 - 1 | | |

The present study investigated the potential of *Lactobacillus casei rhamnosus* strain 35 (LCR35) to improve the results in patients receiving standard HP eradication therapy in Austria.

## MATERIALS AND METHODS

### Study design

This was a pilot, prospective, non-interventional study (NIS) performed at three investigational sites (private practices offering gastroscopies and HP eradication therapy) across Austria. The NIS complied with Directive 2001/20/EC, article 2 and §2a of the Austrian Medical Products Act and was authorized by the Austrian health authorities (AGES - NIS004525). Therefore, an ethics committee approval was not required. All participating patients provided verbal informed consent.

All patients were treated according to the summary of product characteristics (SmPC) of LCR35 (Antibiophilus® powder for oral use, BIOSE, France). HP eradication therapy was initiated as per investigators' discretion and drugs were administered per approved indication (Table 1). No study-specific visits or interventions were required. The participating investigators performed stomach biopsies. Samples were analysed by an independent pathology laboratory. In case a HP infection was confirmed, HP eradication was performed and supplemented by LCR35, 1.5 x $10^8$ CFU, 1-2 times per day at the local investigator's discretion. Patients were advised to take the antibiotics during a meal for better tolerance and LCR35 at least one hour before or after the meal for an improved survival rate of the microbes in the stomach, and/or about two hours after the antibiotic.

At the first control visit at approximately 7 to 14 days after therapy start, patients were asked for occurrence of diarrhoea and other side effects of HP eradication therapy. Diarrhoea was defined as more than 3 stools per day with mushy or watery consistency, following the Bristol stool form scale 5-7 (Lewis and Heaton, 1997). During the final visit approx. 8 weeks after start of therapy, the patients again were asked for diarrhoea and other side effects of HP eradication therapy and a stool sample was taken. These samples were sent to three independent laboratories for confirming the HP eradication by a stool antigen test for HP. All data was collected in case report forms. Histologic examinations after stomach biopsy as well as HP antigen tests were performed independently from the investigators. The involved laboratories were blinded to the fact that the patients were participating in the NIS.

### Eligibility criteria

Adult patients ≥ 18 years of age with a stomach biopsy specimen positive for HP infection providing informed consent were included. Patients with diarrhoea during the week before study start and those using probiotic products during 4 weeks prior to study start were excluded. Table 2 shows a detailed list of inclusion and exclusion criteria.

**TABLE 2. Inclusion and exclusion criteria.**

| |
|---|
| **Inclusion criteria** |
| • adult patients aged ≥ 18 years |
| • stomach biopsy specimen positive for HP infection |
| • Patient informed consent to study participation and anonymous evaluation of the data |
| **Exclusion criteria** |
| • Contraindication to the study medication |
| • Participation in another study |
| • Use of probiotic medical products or food supplements during 4 weeks prior to study start |
| • Diarrhoea during the last week prior to study start |
| • Suspected acute pancreatitis |
| • Patients with jejunum catheter |

### Study objectives

The primary objective was to evaluate the incidence of AAD during HP eradication and LCR35 diarrhoea prophylaxis. The secondary objectives were to determine the HP eradication rate and to evaluate the safety and tolerability of LCR35. Additionally, the present study was intended to gain practical experience for a confirmatory phase III study provided that positive results could be achieved.

### Statistical analysis

The statistical analysis was epidemiological and descriptive in nature. No study hypothesis was tested. All enrolled patients were included in the analysis of incidence of diarrhoea and adverse events, as well as HP eradication rates. Two analysis sets were defined: The full analysis set (FAS) included all eligible and enrolled patients. Patients in the FAS who had discontinued from the study due to any reason were assumed to be non-responders, i.e. having developed diarrhoea and having a HP positive stool test. The completers analysis set (CAS) comprised only those patients with available data at the final visit. The

different antibiotic regimens and LCR35 administration schedules were not analysed separately, because the patient numbers in the respective groups would have been too small.

## RESULTS

### Patient characteristics and demographics

Between March and October 2016, 114 patients presenting with clinical symptoms who had a positive biopsy specimen for HP infection and fulfilled all inclusion criteria were enrolled in three Austrian private practices in Vienna, Schladming, and Mariazell. Two patients were excluded from the analysis, because they did not receive standard antibiotic-based HP eradication therapy: one patient due to the critical medical condition and the other patient due to refusal of antibiotic treatment. Instead, both patients received LCR35 and the PPI omeprazole alone. The analysed study population (n=112) included 42 males (37.5%) and 70 females (62.5%) with a mean age of 52.4 years. At least one concomitant disease was documented in 42 patients (37.5%) and 31 patients (27.9%) received at least one co-medication (Table 3, Supplements 1 and 2). The first and final visits took place in the mean on day 23 and on day 65.3 after therapy start, respectively; 111 patients (99.1%) completed the first visit and 107 patients (95.5%) completed the final visit.

**TABLE 3. Patient characteristics and demographics (N=112).** HP, helicobacter pylori; IQR, interquartile range; SD, standard deviation

| **Characteristic** | |
|---|---|
| Gender, n (%) | |
| Male | 42 (37.5) |
| Female | 70 (62.5) |
| Age, years | |
| Mean (SD) | 52.37 (16.01) |
| Median (IQR) | 54.5 (40, 63) |
| Range | 18-85 |
| Concomitant diseases, n (%) | 42 (37.5) |
| Co-medication, n (%) | 31 (27.9) |

### Incidence of diarrhoea (primary objective)

Diarrhoea was documented in three of the 112 participating patients; two patients reported diarrhoea at the first control and the final visit, one at the final visit only. No episodes of diarrhoea were documented in 104 patients (92.9% FAS). Five patients were lost to follow-up and thus no diarrhoea status was documented. The FAS counting patients lost to follow-up as non-responders, showed an AAD rate of 7.1% (8 in 112 patients). The CAS that used only those patients with available data at the final visit (n=107) showed an AAD rate of 2.8% (Table 4).

**SUPPLEMENT 1. Concomitant diseases (multiple entries possible).** Forty-two patients (37.5 %) had at least one concomitant disease, with a maximum of seven diseases per patient.

| **Concomitant diseases** | **N** |
|---|---|
| Anaemia | 1 |
| Arterial hypertension | 16 |
| Arteriosclerosis | 4 |
| Arthrosis of the fingers | 1 |
| Atrial fibrillation arrhythmia | 1 |
| Ca C. ascendens | 1 |
| Carotis sclerosis | 1 |
| Carotis stenosis | 2 |
| Cervical syndrome | 1 |
| CHD (chronic heart disease) | 2 |
| Cholecystolithiasis | 1 |
| Chronic cephalea | 1 |
| Chronich depression | 1 |
| Chronic venous insufficiency | 3 |
| CKD (chronic kidney disease) | 1 |
| Colon diverticulosis | 1 |
| COPD | 1 |
| Depression | 4 |
| Diabetes mellitus 2 | 3 |
| Essential tremor | 1 |
| Folic acid deficiency | 1 |
| Hashimoto thyroiditis | 1 |
| Hypercholesterolemia | 12 |
| Hyperlipidaemia | 1 |
| Hypertension | 3 |
| Hypothyreosis | 2 |
| Incipient arterial hypertension | 1 |
| Incontinence | 1 |
| Insomnia | 1 |
| Intermittent atrial fibrillation | 2 |
| Latent hypothyreosis with Hashimoto thyroiditis | 1 |
| Latent postoperative hypothyreosis | 1 |
| Lumbar vertebral syndrome | 2 |
| Osteopenia | 3 |
| Osteoporosis | 2 |
| Pancreatic insufficiency | 2 |
| Paroxysmal supraventricular tachycardia | 1 |
| Proctitis ulcerosa | 1 |
| Prostate hypertrophy | 1 |
| Reflux esophagitis | 1 |
| Restless legs syndrome | 1 |
| Sigma diverticulosis | 1 |
| Status post pulmonary embolism | 1 |
| Struma | 2 |
| Vitamin D deficiency | 1 |
| **Total** | **93** |

**SUPPLEMENT 2. Concomitant medications (multiple entries possible).** Thirty-one patients (27.9 %) had at least one concomitant medication, with a maximum of eight medications per patient.

| Concomitant medication (s) | N |
|---|---|
| Acenocoumarol | 2 |
| Acetylsalicylsäure | 5 |
| Amlodipin | 4 |
| Amlodipin, Valsartan & Hydrochlorothiazid | 1 |
| Asasantin | 1 |
| Atenolol | 1 |
| Atorvastatin | 3 |
| Bisoprolol | 3 |
| Carvedilol | 2 |
| Coagulation factor Xa | 1 |
| Doxazosin | 2 |
| Duloxetin | 1 |
| Empagliflocin | 1 |
| Enalapril | 1 |
| Finasterid | 1 |
| Flavonoids | 2 |
| Folic acid | 1 |
| Gliglacid | 1 |
| Horse chestnut extract | 1 |
| Imidapril | 1 |
| Isosorbit-Mononitrat | 1 |
| Ivabradin | 1 |
| Levothyroxin | 5 |
| Linagliptin | 1 |
| Lisinopril | 1 |
| Mefenamic acid | 1 |
| Meloxicam | 1 |
| Mesalazin | 1 |
| Metamizol | 1 |
| Metformin | 1 |
| Moxonidin | 1 |
| Nebivolol | 1 |
| Olmesartan | 2 |
| Olmesartanmedoxomil & Hydrochlorothiazid | 1 |
| Olmesartanmedoxomil, Amlodipin & Hydrochlorothiazid | 1 |
| Passion flower extract | 1 |
| Phenprocoumon | 1 |
| Prasugrel | 1 |
| Propranolol | 1 |
| Ramipril | 4 |
| Rosuvastatin | 2 |
| Simvastatin | 10 |
| Spironolacton | 1 |
| Thrombo-ASS | 3 |
| Tizanidin | 1 |
| Tolterodin | 1 |
| Trazodon | 6 |
| Vitamin D3 (Cholecalciferol) | 9 |
| **Total** | **96** |

**TABLE 4. Rates of antibiotics-associated diarrhoea and HP eradication.** FAS, full analysis set: included all eligible and enrolled patients and those who had discontinued from the study due to any reason were assumed to be non-responders, i.e. having developed diarrhoea and having a HP positive stool test. CAS, completers analysis set: comprised only those patients with available data at the final visit.

| | n (%) |
|---|---|
| **AAD rate** | |
| FAS (N=112) | 8 (7.1) |
| CAS (N=107) | 3 (2.8) |
| **HP eradication rate** | |
| FAS (N=112) | 102 (91.1) |
| CAS (N=107) | 102 (95.3) |

## Secondary objectives

### Rate of HP eradication

The proportion of patients receiving each of the indicated HP eradication schemes is shown in Table 1. The vast majority of patients received sequential standard triple therapy consisting of PPI 2x1 units, amoxicillin 2x1 g, followed by PPI 2x1 units, levofloxacin 2x500 mg, metronidazole 2x500 mg with additional courses of LCR35 during the whole antibiotics therapy. A HP negative stool antigen test result at final visit was reported for 102 of 112 patients (91.1%), whereas 5 patients had a positive HP test. HP eradication rates are shown in Table 4.

### Adherence to treatment

Out of 112 patients initiating treatment, 107 adhered to the planned sequence and duration of antibiotics therapy (95.5%). No documentation of a first control visit is available for one patient; five patients were lost to follow-up by the time of the final visit; it is unknown whether these patients completed the full course of their antibiotics treatment.

### Adverse events

Seven of 112 patients (6.3%) reported adverse events (including diarrhoea), which mainly were of gastrointestinal nature.

## DISCUSSION

In Austria, the medicinal product Antibiophilus® with the active ingredient LCR35, is approved for the treatment of diarrhoea of different origins, including the treatment of AAD and diarrhoea caused by radiation therapy (Bundesamt für Sicherheit im Gesundheitswesen 2015). The present pilot study investigated the efficacy of 1.5 x $10^8$ CFU of LCR35 to prevent AAD (primary endpoint) when administered in combination with a standard HP eradication scheme, and to assess the rate of HP eradication, as well as the safety and tolerability of LCR35 as secondary endpoints. According to the FAS, this study reports an AAD rate of 7.1% (8 of 112 patients) and a HP eradication rate of 91.1% (102 of 112 patients). Five patients were lost

to follow-up and were considered non-responders as per the definition of the FAS. The present study did not have a suitably designed control arm with patients not receiving LCR35 and as a consequence, the effect size of AAD prevention induced by the supportive administration of LCR35 cannot be determined from this study, nor can it be concluded that LCR35 had contributed to the rate of HP eradication found. However, strong arguments to support both claims can be made and a hypothesis can be generated, which needs to be confirmed in future randomized controlled trials.

Probiotics, especially certain strains of Lactobacillus ssp., have shown inhibitory activity against HP *in vitro* (Pinchuk et al., 2001; Myllyluoma et al., 2008) and *in vivo* (Lievin-Le Moal and Servin, 2014), may colonize the stomach (Delgado et al., 2014), and even increase HP eradication rates (Lievin-Le Moal and Servin, 2014; Homan and Orel, 2015; McFarland et al., 2016). Probiotics may eradicate HP via several immunological and non-immunological antibacterial mechanisms, for instance by modifying host immune responses or by secreting antibacterial substances such as lactic acid, short chain fatty acids, hydrogen peroxide and bacteriocins (Homan and Orel, 2015). Mechanisms by which probiotics interfere with HP adherence to host mucosa have also been suggested (Homan and Orel, 2015). However, a difference in efficacy was reported for different strains of probiotics. A meta-analysis of 33 randomized, controlled trials involving 4459 patients showed that of the investigated probiotic strains, only *Lactobacillus acidophilus*, *Lactobacillus casei* DN-114001, *Lactobacillus gasseri*, and *Bifidobacterium infantis* 2036 increased eradication rates in antibiotic therapies with eradication rates <80% (Dang et al., 2014). Another meta-analysis showed similar results (Lievin-Le Moal and Servin, 2014). Nevertheless, the existing study results were strong enough for the Maastricht V/Florence Consensus Report (Malfertheiner, Megraud et al. 2017) as well as the German S2k guidelines (Fischbach et al., 2016) to recommend the use of probiotics for reduction of AAD and increasing eradication rates and adherence to treatment, but without any special recommendation for specific strains. For patients contraindicated to antibiotics or who refuse antibiotic therapy, a combination of a probiotic such as LCR35 and a PPI may thus be an alternative therapeutic option. Due to their low cost (< 1 Euro/day), probiotics may also be an option in low-income countries with commonly high resistance rates. There is thus a strong need to investigate alternatives, such as combined antibiotic and probiotic therapies. When gastric pH raised above 4.0 by PPIs, *Lactobacillus* spp., *Streptococcus* spp., and other gastric bacteria proliferate (Freedberg et al., 2014). In the present study, two patients were excluded from analysis, because they had not received an antibiotic standard therapy, but LCR35 and a PPI alone. Both patients were HP negative at the end of their treatment.

The question to be answered is, if LCR35 belongs to the effective strains of probiotics in HP eradication therapy. The present study is the first step in a research program to answer this question. Of course, there are some limitations, mainly the uncontrolled, open-label design within the framework of a pilot study. The study was performed under daily clinical practice conditions of specialist practices, therefore different HP eradication schemes and different LCR35 administrations schedules were used. No resistance analysis was performed at baseline and no documentation of the rate of treatment experienced patients is available. In addition, there was no control group. Adverse events were documented from patients' memory alone and are commonly underreported in this type of study. However, the high eradication rate of >90% is based on the results of external, independent laboratories, without subjective influence of the treating physicians or treated patients. Taken together, the results of this pilot study encourage for a further investigation in a randomized, controlled trial.

## CONCLUSIONS

This study supports previous reports that LCR35 may have beneficial effects as a supplement to antibiotic-based HP eradication therapy. Apart from their efficacy in preventing AAD, probiotics have been shown to exercise antibacterial effects of their own and may thus be used as an adjunct to antibiotics in times of increasing resistance. Their potential to reduce side effects of antibiotic therapies may increase patient adherence, which in turn may halt or decelerate the development of HP resistance to antibiotics. LCR35 is a well characterized probiotic medicinal product with an excellent safety profile. Further investigations in a randomized, controlled trial are needed to determine its role in HP eradication.

## CONFLICT OF INTEREST DISCLOSURE

This was an investigator-initiated study sponsored by Babak Bahadori. The study investigators did not receive financial compensation for any part of their work on this study. Germania Pharmazeutika GmbH, Vienna, Austria, provided financial support for data management and statistical analysis performed by Bioconsult GmbH and provided the study medication. Elisabeth Uitz and Kathayoun Tonninger-Bahadori declare to have no conflicts of interest. Karl Nekrep is an employee of Germania Pharmazeutika GmbH. Babak Bahadori received lecture fees from Germania Pharmazeutika GmbH in 2015.

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BB and KN designed the study and prepared the study protocol. EU, KTB, and BB collected patient data. All authors were involved in the interpretation of the analyses. All authors were involved in critical revision of the manuscript for important intellectual content. All authors approved the final manuscript. The authors wish to thank all participating patients and laboratories for their contributions. Dr. Guenther Nirnberger (Bioconsult GmbH, Breitenfurt, Austria) provided

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