Congestive Heart Failure

Stimulating autoantibodies directed against the cardiac β₁-adrenergic receptor predict increased mortality in idiopathic cardiomyopathy

Stefan Störk, MD, PhD,a Valerie Boivin, PhD,b Rüdiger Horf, MD,a Lutz Hein, MD,b Martin J. Lohse, MD,b Christiane E. Angermann, MD,a and Roland Jahns, MD,a,b Würzburg, Germany

Background The aim of this study was to estimate the independent and incremental prognostic value of the presence of stimulating autoantibodies directed against the human β₁-adrenergic receptor (anti–β₁-AR) in patients with chronic heart failure.

Methods One hundred five antibody-typed chronic heart failure patients with dilated cardiomyopathy (DCM, n = 65) or ischemic cardiomyopathy (ICM, n = 40) were prospectively followed for 10.7 ± 2.5 years. Information on all-cause and cardiovascular mortality was collected throughout the observation period.

Results Stimulating anti–β₁-AR were prevalent in 26% (17/65) of patients with DCM and 13% (5/40) with ICM. All-cause mortality in antibody-positive patients was 65% in those with DCM and 80% in those with ICM, and in antibody-negative patients 44% and 49%, respectively. In univariate and multivariable Cox regression analysis (P < .05), presence of stimulating anti–β₁-AR was associated with increased all-cause and cardiovascular mortality risk in DCM but not in ICM. Information on antibody status improved the prognostic capacity in models containing already extensive information on clinical profile, Holter electrocardiography, and invasive hemodynamic measurements (area under the receiver operating characteristic curve, 0.91; 95% confidence interval, 0.85-0.97; P < .05 for increase in receiver operating characteristic area).

Conclusion The presence of stimulating anti–β₁-AR autoantibodies independently predicts increased all-cause and cardiovascular mortality risk in DCM conferring incremental prognostic value in addition to established risk predictors. Our data indicate a clinical relevance of stimulating anti–β₁-AR in DCM and encourage further research into antibody-directed strategies as a therapeutic principle. (Am Heart J 2006;152:697-704.)

Current hypotheses on exogenous causes of idiopathic dilated cardiomyopathy (DCM) focus on chronic myocarditis1 and on primary abnormalities in the immune system.2,3 In both cases, cardiac tissue injury is thought to be mediated mainly by cytokines and/or heart-specific autoantibodies.3,5 Evidence is growing that autoantibodies that bind to and activate the human β₁-adrenergic receptor (β₁-AR), that is, stimulating autoantibodies, may be involved in the initiation and progression of DCM.5,6 Specifically, antibodies that target the functionally relevant second extracellular receptor domain (ECII) may be particularly relevant.7 Recently, we were able to show that rats immunized with β₁-ECII (100% sequence identity human/rat) developed both stimulating anti–β₁-ECII antibodies and progressive DCM.8 Subsequent transfer of anti–β₁-ECII antibodies from immunized to healthy rats (to mimic autoantibodies) also transferred the disease and hence provided direct evidence for a cause-and-effect relationship between stimulating anti–β₁-AR antibodies and DCM.8,9 In humans, anti–β₁-ECII autoantibodies have been detected in 26% to 95% of patients with DCM and in about 10% of patients with ischemic cardiomyopathy (ICM), depending on the screening strategy.6,10-14 Previous studies reported that DCM patients positive for anti–β₁-ECII autoantibodies exhibited significantly poorer left ventricular function,12 a higher prevalence of serious ventricular arrhythmias,14,15 and a higher incidence of sudden cardiac death,14 when compared with antibody-negative subjects. The aim of the present study was to estimate the independent prognostic value of autoantibodies that recognize and stimulate the native human β₁-AR for predicting mortality risk in DCM and
ICM over a follow-up period of more than 10 years. The incremental prognostic utility of the antibody status for all-cause and cardiovascular mortality was assessed against the background of a comprehensive clinical, electrocardiographic, and hemodynamic patient profile.

**Methods**

**Study population and design**

One hundred five patients with chronic heart failure were recruited for this prospective cohort study during routine heart catheterization if their left ventricular diastolic volume was >110 mL/m² and ejection fraction measured by ventriculography was <55%, as described previously. DCM (n = 65) was diagnosed when coronary artery disease was excluded by angiography, and no exposure to cardiotoxic substances, history of myocarditis, or myocardial involvement in systemic disease was evident from the medical records. ICM (n = 40) was diagnosed when a significant stenosis (>75%) of one or more of the main coronary arteries was ascertained by angiography. All patients were examined under stable hemodynamic conditions at the time of sample acquisition (1988-1995). At that time, none of the patients received β-blocking agents or sympathomimetic drugs with the exception of 3 patients with ICM who were on sotalol 80 mg BID. Vital status and cause of death in study participants (100% complete) were ascertained up to December 2003 by contacting the patients' general practitioner. Cardiovascular death was assumed if one of the following reasons of death was given: myocardial infarction, cerebrovascular event, decompensated heart failure, or sudden death (defined as unexpected death within 1 hour of the onset of acute symptoms or unwitnessed death, ie, during sleep, in a patient known to have been well within the preceding 24 hours). The study was approved by the Medical Ethics Committee of the University of Würzburg.

**Screening strategy for stimulating β₁-receptor autoantibodies**

Presence of functionally active autoantibodies that recognize and stimulate native human β₁-AR was detected in 2 steps as previously described in detail. Briefly, in the first step, patient immunoglobulin G (IgG) was tested for binding to synthetic peptide-analogues of selected domains of the human β₁- and β₂-AR by enzyme-linked immunosorbsent assay and dot blotting (Figure 1). In the second step, positive IgG fractions were assayed for binding to native human β₁- and β₂-AR expressed in the membrane of Sf9 insect cells (immunofluorescence microscopy). IgG fractions staining β₁-AR in Sf9 cells (with or without β₂-AR cross-reactivity) were then tested for their effects on intracellular cAMP generation and protein kinase A activity in Chinese hamster fibroblasts (CHW cells) stably expressing human β₁-AR (Figure 1).

**Data analysis**

Data are presented as mean (SD) or absolute numbers (percent), as appropriate. Nonparametric tests were applied to compare groups of patients. For continuous data, the Mann-Whitney U test was used, and for categorical data, the χ² test. The association of antibody positivity with all-cause and cardiovascular mortality was estimated by Cox proportional hazards regression. After identification of univariate predictors using a liberal P value < .10, multivariate modeling was used to evaluate the independent relationship between these parameters and the respective outcome. Determinants were then backward eliminated from the multivariate model if the change in coefficient was < 20%. All variables left out in the previous step were then reentered one by one to assess their additional contribution to the model. Results in prognostic models are expressed as hazard ratio (HR) with 95% confidence interval (CI). In survival plots, curves were estimated by use of the mean values of covariates in respective Cox regression models. To estimate the prognostic capacity of different sets of predictors (models), receiver operating characteristic (ROC) curves were constructed. The area under the curve was
calculated using the nonparametric trapezoidal rule, with its standard error and 95% CI. Differences in the discriminative value between models were estimated by differences in ROC area, taking into account the correlation between models as they were based on the same cases. All \( P \) values are reported 2-sided. Statistical analysis was performed using SPSS 11.5.1 (SPSS Inc, Chicago, IL).

### Results

Clinical, hemodynamic, and Holter electrocardiographic characteristics at baseline

The baseline characteristics of this predominantly male study population are given in Table I. Patients are grouped according to heart failure etiology. In addition, patients with DCM were subdivided according to antibody status. Age and sex distribution did not differ between groups. There was a trend for less pronounced clinical heart failure symptoms in patients with ICM compared with patients with DCM (55% vs 77% New York Heart Association [NYHA] III-IV; \( P = .061 \)). Within the DCM group, heart failure symptoms were more severe in antibody-positive patients (94% vs 71% NYHA III-IV; \( P = .034 \)). At baseline, more patients with DCM received angiotensin converting enzyme (ACE) inhibitors (80% vs 55%) and/or cardiac glycosides (57% vs 30%), whereas nitroglycerine use was more...
frequent in patients with ICM (98% vs 32%; all \( P < .01 \), respectively). No significant differences in medication were noted between antibody-positive and antibody-negative patients with DCM (Table I). Hemodynamic parameters were almost identical between patients with ICM and patients with DCM (Table I). However, left ventricular function was more compromised in antibody-positive patients with DCM as indicated by a higher heart rate along with lower values for left ventricular contractility (+\( dp/dt \)), relaxation (−\( dp/dt \)), ejection fraction, and cardiac index (all \( P < .05 \)). Analysis of Holter electrocardiographic readings (obtained at baseline) revealed no significant differences between patients with ICM and patients with DCM regarding the prevalence of atrial fibrillation, left or right bundle branch block, and of supraventricular premature capture beats (Table I). By contrast, prevalence of first-degree atrioventricular conduction delay (26% vs 8%) and frequency of ventricular premature capture beats (VPCs) including runs of \( \geq 4 \) VPCs (35% vs 13%) was higher in DCM (\( P < .05 \), respectively). In DCM, no differences between antibody-positive and antibody-negative patients were observed with respect to the absolute number of VPCs per 24 hours or the length and frequency of ventricular runs. However, Lown class 0-2 arrhythmia occurred less often (6% vs 23%), but Lown class 4 arrhythmia more often (82% vs 62%), in antibody-positive versus antibody-negative patients (\( P < .05 \), respectively; Table I).

### All-cause and cardiovascular mortality

According to our stringent screening procedure (Figure 1), functionally active autoantibodies able to recognize and stimulate native human \( \beta_1 \)-AR were prevalent in 26% (17/65) of patients with DCM and 13% (5/40) of patients with ICM.\(^{12} \) During a mean follow-up period of 10.7 ± 2.5 years for survivors, all-cause (cardiovascular) mortality in antibody-positive patients was 65% (59%) with DCM and 80% (80%) with ICM, whereas in antibody-negative patients, mortality was 44% (35%) in DCM and 49% (46%) in ICM, respectively. In univariate analyses, antibody-positive status was predictive in patients with DCM only (all-cause mortality—HR, 2.00; 95% CI, 1.21-4.19; \( P = .022 \); cardiovascular mortality—HR, 2.24; 95% CI, 1.10-4.67; \( P = .024 \)).

**Table II.** Univariate and multivariate predictors of all-cause mortality in patients with dilated and ICM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HR</th>
<th>95% CI</th>
<th>( P )</th>
<th>HR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM, n = 65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti–( \beta_1 )-AR positive</td>
<td>2.00</td>
<td>1.21-4.19</td>
<td>.022</td>
<td>3.76</td>
<td>1.39-7.89</td>
<td>.009</td>
</tr>
<tr>
<td>NYHA functional class, per class</td>
<td>2.39</td>
<td>1.10-5.14</td>
<td>.026</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Heart rate, per 10 beats/min</td>
<td>1.38</td>
<td>1.07-1.78</td>
<td>.013</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chronic use of nitroglycerine</td>
<td>2.59</td>
<td>1.26-5.33</td>
<td>.010</td>
<td>4.59</td>
<td>1.91-11.04</td>
<td>.001</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>.96</td>
<td>.93-99</td>
<td>.009</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LV end-diastolic pressure, per mm Hg</td>
<td>1.05</td>
<td>1.00-1.11</td>
<td>.043</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac index, per L min ( -1 ) m ( -2 )</td>
<td>.56</td>
<td>.28-1.09</td>
<td>.086</td>
<td>.13</td>
<td>.02-6.7</td>
<td>.015</td>
</tr>
<tr>
<td>Contractility, per 100 mm Hg/s</td>
<td>.87</td>
<td>.77-98</td>
<td>.025</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Relaxation, per 100 –mm Hg/s</td>
<td>.81</td>
<td>.70-94</td>
<td>.006</td>
<td>.80</td>
<td>.63-98</td>
<td>.049</td>
</tr>
<tr>
<td>PCWP, per mm Hg</td>
<td>1.05</td>
<td>1.01-1.09</td>
<td>.008</td>
<td>1.09</td>
<td>1.03-1.15</td>
<td>.003</td>
</tr>
<tr>
<td>ICM, n = 40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>2.15</td>
<td>0.91-5.09</td>
<td>.086</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>2.54</td>
<td>0.98-6.61</td>
<td>.055</td>
<td>7.57</td>
<td>1.73-32.11</td>
<td>.007</td>
</tr>
<tr>
<td>Diuretic</td>
<td>3.26</td>
<td>1.19-8.95</td>
<td>.022</td>
<td>5.89</td>
<td>1.26-17.47</td>
<td>.024</td>
</tr>
<tr>
<td>Digitalis</td>
<td>2.19</td>
<td>0.92-5.23</td>
<td>.078</td>
<td>3.50</td>
<td>1.24-9.88</td>
<td>.018</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>0.97</td>
<td>0.94-1.00</td>
<td>.059</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac index, per L min ( -1 ) m ( -2 )</td>
<td>0.37</td>
<td>0.18-0.77</td>
<td>.007</td>
<td>0.64</td>
<td>0.24-0.92</td>
<td>.047</td>
</tr>
<tr>
<td>Contractility, per 100 mm Hg/s</td>
<td>0.86</td>
<td>0.75-1.00</td>
<td>.046</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Relaxation, per 100 –mm Hg/s</td>
<td>0.86</td>
<td>0.72-1.02</td>
<td>.089</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Absolute arrhythmia</td>
<td>2.42</td>
<td>0.93-6.29</td>
<td>.069</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Left bundle branch block</td>
<td>2.01</td>
<td>0.85-4.74</td>
<td>.113</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Multivariate analysis** | | | | | | |
| Anti–\( \beta_1 \)-AR positive | 2.00 | 1.21-4.19 | .022 | 3.76 | 1.39-7.89 | .009 |
| NYHA functional class, per class | 2.39 | 1.10-5.14 | .026 | – | – | – |
| Heart rate, per 10 beats/min | 1.38 | 1.07-1.78 | .013 | – | – | – |
| Chronic use of nitroglycerine | 2.59 | 1.26-5.33 | .010 | 4.59 | 1.91-11.04 | .001 |
| LV ejection fraction, % | .96 | .93-99 | .009 | – | – | – |
| LV end-diastolic pressure, per mm Hg | 1.05 | 1.00-1.11 | .043 | – | – | – |
| Cardiac index, per L min \( -1 \) m \( -2 \) | .56 | .28-1.09 | .086 | .13 | .02-6.7 | .015 |
| Contractility, per 100 mm Hg/s | .87 | .77-98 | .025 | – | – | – |
| Relaxation, per 100 –mm Hg/s | .81 | .70-94 | .006 | .80 | .63-98 | .049 |
| PCWP, per mm Hg | 1.05 | 1.01-1.09 | .008 | 1.09 | 1.03-1.15 | .003 |

This table lists the univariately predictive variables for all-cause mortality obtained from separate analyses for patients with DCM and patients with ICM. According to the multivariate analysis, significant predictors for cardiovascular mortality were age, left ventricular ejection fraction, pulmonary capillary wedge pressure, and amiodarone intake (detailed data not included in Table II).
shown). By contrast, in patients with ICM, only cardiovascular medication and cardiac index, but not antibody status, predicted mortality in multivariate regression (Table II). Figure 2 depicts the adjusted survival curves for all-cause and cardiovascular mortality in antibody-positive and antibody-negative patients with DCM and patients with ICM, adjusted for the respective multivariable predictors.

Incremental prognostic value of antibody-positive status in the prediction of all-cause and cardiovascular mortality

The incremental prognostic value to predict all-cause and cardiovascular mortality risk in a clinical workup situation was estimated by comparing the area under ROC curves derived from successively added risk marker sets (Figure 3). The addition of information on medication, Holter electrocardiogram, and hemodynamic measurements significantly increased the ROC area at each step. Information on antibody status further increased the area under ROC curve from 0.85 to 0.91 for all-cause mortality and from 0.86 to 0.90 for cardiovascular mortality (both \( P < .05 \)).

Discussion

The main finding of this prospective follow-up study in patients with heart failure is that autoantibodies that bind to and stimulate the human \( \beta_1 \)AR are independent predictors of all-cause and cardiovascular mortality in DCM. Antibody status confers incremental information on top of a detailed clinical, electrocardiographic, and hemodynamic patient profile. Our findings underscore the clinical relevance of stimulating anti-\( \beta_1 \)AR autoantibodies in DCM, particularly if viewed together with their recently demonstrated pathogenetic potential in animals, and encourage antibody-directed strategies in antibody-positive patients as a therapeutic principle. Alternative therapeutic strategies may comprise elimination of stimulating anti-\( \beta_1 \)AR autoantibodies by selective immunoadsorption and/or...
induction of immune tolerance via direct targeting of the anti-β1-AR producing B-cells.22

Cardiac disease and heart-specific antibodies

Alterations in humoral and cellular immunity have been described in both ischemic and non-ICM.5,12,25 Idiopathic DCM has been linked to immune responses associated with or induced by infections (ie, cardiotropic viruses, bacteria, or parasites 5,24,25) because a substantial number of DCM patients has circulating cross-reacting antibodies and/or autoantibodies against a wide panel of cardiac antigens. These include membrane proteins,5,6,11,12,28 mitochondrial proteins,29,30 and/or myocyte structural proteins.25,31 Genetic factors may also be involved in the immune pathogenesis of DCM, either as contributors to the susceptibility to immunologic factors or as determinants of myocyte functional and structural changes that characterize the phenotypic expression of the disease.52

So far, the pathophysiologic relevance of most cardiac autoantibodies is far from clear. First, low titers of autoantibodies to various housekeeping antigens can also be detected in healthy subjects as part of the natural immunologic repertoire.27 However, we have previously shown that the prevalence of stimulating anti–β1-AR autoantibodies in healthy individuals was rather low (<1%) when using the screening strategy described above.12 Second, under physiologic conditions, most cardiac antigens remain hidden from the immune system, at least if localized intracellularly. Functional β1-AR, however, are readily accessible targets localized on the cell surface. Third, the harmful potential of an autoantibody depends on the functional importance of its target. Regarding this aspect, we recently demonstrated in a rat model of cardiomyopathy that induction or repetitive isogenic injection of stimulating anti-β1-AR antibodies caused adverse long-term effects on myocardial force development and relaxation by interaction with the cardiac β1-AR.8 The findings of the present study in conjunction with the available experimental evidence suggest that antibodies directed against cell surface located key constituents and in particular autoantibodies that bind to and stimulate cardiac β1-AR may play an important role also in the initiation and/or progression of human DCM.19

Mortality and stimulating anti–β1-AR autoantibodies

To our knowledge, this is the first study demonstrating over a follow-up period of more than 10 years that presence of stimulating anti–β1-AR autoantibodies is independently associated with an about 3-fold increase in all-cause and cardiovascular mortality risk in DCM. This adds evidence to the results from a previous report on 104 patients with DCM in which the authors described a higher sudden cardiac death rate associated with autoantibody-positivity in patients observed for a mean follow-up period of 2.5 years.14 In the present study, information on patients’ antibody status further increased the prognostic capacity beyond that of extensive clinical, electrocardiographic, and hemodynamic data. This single parameter increased the ROC area by 6% against the background of 24 variables thought to be relevant for risk prediction in this patient population (Figure 3). In contrast, such an association was absent for patients with ICM. This was not unexpected because mortality risk in ICM is mainly explained by a well-characterized panel of nonimmunologic risk factors. However, because the number of autoantibody-positive patients with ICM in our study was small, we cannot exclude with certainty that stimulating anti–β1-AR autoantibodies would not influence prognosis also in ICM.

Cardiac arrhythmias and stimulating anti–β1-AR autoantibodies

Based on their proposed sympathomimetic activity,8 stimulating anti–β1-AR autoantibodies has also been claimed to modify electrophysiologic properties of the normal heart, probably by increasing the L-type calcium current.13 The sustained calcium influx in the presence of these autoantibodies was found to activate the sodium/calcium exchanger in both human and animal cardiomyocytes, thereby inducing electric instability of the heart.33 Concordant with these in vitro experiments, recent clinical observations indicate that the presence of anti-β1-AR autoantibodies in patients with DCM is associated with both occurrence of more severe ventricular arrhythmia and sudden cardiac death.14,15 In agreement with these observations, we found a higher prevalence of VPCs and Lown class 3-4 arrhythmia in antibody-positive compared with antibody-negative DCM patients (see Table I). However, we did not find an increase in nonsustained or high-risk ventricular tachycardia (ie, lasting ≥3 seconds or heart rate ≥165 beats/min) in antibody-positive subjects, and the documented rhythm disturbances were not predictive of an increased mortality risk in multivariate analyses. Differences in the screening modalities aiming at the detection of anti–β1-AR autoantibodies may account for these slightly diverging results. Previously, we have shown that only about half of the enzyme-linked immunosorbent assay–defined autoantibodies were able to bind to native cell surface located β-AR. Only this antibody fraction was also able to activate recombinant human β1-AR expressed at physiologic levels in the membrane of CHW cells (as determined by antibody-dependent increases in cellular cAMP generation and PKA activity).12 We therefore advocate cell systems presenting the target in its natural conformation to screen for functionally relevant β-receptor autoantibodies (see Methods and Figure 1).
Limitations and strengths

Because our cohort was sampled before β-blockade was established as a first line treatment in the guidelines for heart failure therapy, we were able to study β-blocker-naïve patients with DCM. Initiation of β-blockade in the last 2 years of the observation period may have affected outcome but, if anything, would have led to an understimation of the true antibody-mediated adverse effect on mortality risk. Furthermore, our sample size was limited compared with the number of baseline variables among which univariate predictors had to be sought. We therefore used a conservative approach to variable selection using the change-in-estimate criterion. Strengths of this investigation comprise the thorough collection of a broad spectrum of baseline characteristics in both patients with DCM and patients with ICM, the stringent methodology to identify anti-β1-AR autoantibodies, and the long duration of follow-up with complete verification of end points.

Conclusion

In conclusion, we observed an about 3-fold increase in all-cause and cardiovascular mortality risk associated with the presence of stimulating anti-β1-AR autoantibodies in DCM, whereas this association was absent in ICM. Anti-β1-AR antibody status confers incremental prognostic value in addition to a comprehensive clinical, electrocardiographic, and hemodynamic patient profile.

Our findings underscore the clinical relevance of anti-β1-AR autoantibodies in DCM and encourage further research in the field of antibody-directed strategies as a therapeutic principle.

References


Access to American Heart Journal Online is reserved for print subscribers!

Full-text access to American Heart Journal Online is available for all print subscribers. To activate your individual online subscription, please visit American Heart Journal Online, point your browser to http://www.ahjonline.com, follow the prompts to activate your online access, and follow the instructions. To activate your account, you will need your subscribe account number, which you can find on your mailing label (note: the number of digits in your subscriber account number varies from 6 to 10). See the example below in which the subscriber account number has been circled:

Sample mailing label

FEB00 J004 C: 1 234567-80 SJ P1
J. H. DOE, MD
531 MAIN ST
CENTER CITY, NY 10001-001

Personal subscriptions to American Heart Journal Online are for individual use only and may not be transferred. Use of American Heart Journal Online is subject to agreement to the terms and conditions as indicated online.