Mortality in inherited cardiac diseases: directing care in affected families
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Chapter 6

The effect of ascertainment bias on estimates of patient mortality in inherited cardiac diseases

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Submitted
ABSTRACT

Objective
To evaluate the effect of ascertainment bias on survival in three different inherited cardiac diseases caused by a founder mutation in patients with idiopathic ventricular fibrillation (IVF), SCN5A overlap syndrome, and arrhythmogenic cardiomyopathy (ACM), since ascertainment bias can influence survival estimates greatly.

Patients and methods
We collected mortality data from mutation carriers of IVF, SCN5A overlap syndrome and ACM over 2-10 years of ongoing clinical/genetic cascade screening.

Results
The median age of survival in the IVF patients increased from 44.6 years in 2008 (n=60, 95%-CI: 36.8-52.4 years) to 68.2 years in 2012 (n=235, 95%-CI: 64.6-71.7 years; p<0.001). In the SCN5A overlap syndrome, survival increased from 56.1 years in 1999 (n=86, 95%-CI: 48.0-64.2 years) to 69.7 years in 2009 (n=197, 95%-CI: 61.3-78.2 years; p=0.049). In ACM patients, the median age of survival increased from 63.5 years in 2010 (n=89, 95%-CI: 59.1-68.0 years) to 65.2 years in 2012 (n=370, 95%-CI: 62.0-68.3 years; p= 0.046).

Conclusion
The median age of survival in three different cardiac diseases with an established pathogenic substrate significantly increased once cascade screening began in the years following the first publication that elucidated the discovery of the disease-susceptibility gene/mutation. This underscores the direct and negative influence of ascertainment bias on survival forecasts and the importance of ongoing clinical/genetic follow-up to establish the most accurate disease prognosis for genetically-mediated heart disease.
INTRODUCTION

Since the 1990s, the molecular genetic basis for many inherited diseases has been elucidated. Once the novel causative gene (mutation) is discovered in patients, the first publications in established medical journals contain the clinical and mortality data of the patients’ and families’ that led to the gene/mutation discovery. Not surprisingly, the patients and families described in these gene discovery publications often comprise severely affected patients and relatives in whom gene identification strategies were promising and eventually turned out to be successful. Therefore, the sentinel genotype-phenotype associations in general and survival forecasts in particular could be subject to ascertainment bias.

In the years following the initial identification of the causative gene, genetic testing of other relatives can be performed (genetic cascade screening) and new families with the same gene defect are detected. However, extended data on these newly identified probands and mutation carriers may never be submitted or published. This is because cascade screening studies are either not carried out (due to poor accessibility to genetic services in some countries, for example) or because the publication impact of an already published disease-susceptibility gene/mutation may be deemed too low by the authors or the journal. This may lead to a persistence of the effect of the ascertainment bias on risk estimates in literature. As a consequence, a possible overestimation of the risks in gene/mutation-positive individuals is maintained in clinical practice.

This undesirable and misleading effect of ascertainment bias on phenotypic characteristics in patients with an inherited disease have been observed. For example, in initial studies on hypertrophic cardiomyopathy (HCM) or Brugada syndrome, the annual mortality and event rates appeared much higher than in later, larger studies with more unselected patients.\(^1,2\) The occurrence of ascertainment bias has also been reported for cancer risk estimates.\(^3,4\)

In order to examine this effect directly, and in greater detail, we analyzed mortality data from mutation carriers over 2-10 years of ongoing cascade screening in large families and newly detected families with three different autosomal dominantly inherited cardiac diseases caused by a founder mutation: idiopathic ventricular fibrillation (IVF), SCN5A overlap syndrome, and arrhythmogenic cardiomyopathy (ACM). We identified new mutation positive probands and a large number of genetically at-risk relatives through genetic cascade screening in the years following the original discovery of the causative gene defect in the proband. This process of genetic testing and cascade screening of probands and relatives is incorporated into the Dutch health care system and allowed us to perform this unique study which quantifies precisely for the first time the negative effect of ascertainment bias on mortality.
METHODS

We collected mortality data (data of birth and death) from proven, obligate, and putative mutation carriers over years of ongoing cascade screening, in families with three different cardiac diseases caused by a founder mutation. Our study included carriers of a risk haplotype (suggestive for a founder mutation) for IVF on chromosome 7q36 harboring the DPP6 gene, carriers of the p.1795insAsp founder mutation in the SCN5A gene causing a SCN5A overlap syndrome, and carriers of the ACM-causing p.Arg14del founder mutation in PLN-encoded phospholamban. We included all proven mutation carriers of these three founder mutations, all obligate carriers, and all first-degree relatives of carriers with unexplained sudden cardiac death (SCD) under the age of 50 years (putative carriers) for our survival analyses.

Starting point in our survival analyses was birth. An event in our survival analyses was death and as equivalents for death, heart transplantation (only for the PLN founder mutation), documented ventricular fibrillation, and appropriate discharge of an implantable defibrillator (ICD). IVF and ACM mutation carriers were censored at last follow-up when alive. In case of the SCN5A overlap syndrome, censoring was at the time DNA diagnostics was performed since treatment with a pacemaker could have been started then. For this specific SCN5A mutation, bradyarrhythmias are believed to be the cause of death and hence pacing is believed to prevent SCD. Because pacing is not registered by the pacemaker, possible cardiac events that required pacing cannot be registered, unlike in an ICD where an appropriate shock is registered. Some patients died before DNA diagnostics was performed (obligate carriers) or received a pacemaker before DNA diagnostics was performed, based on their phenotype. Those patients were censored at the date of pacemaker implantation.

Data were analyzed with SPSS version 19.0.1 for Windows (SPSS Inc., Chicago, Illinois). We compared median age of survival of the cohort at the moment of the discovery/publication of the genetic defect with the median age of survival in the same cohort extended with data derived from subsequently newly detected probands and (obligate and putative) mutation carriers at the end of the follow-up period.

Cumulative survival analyses were performed using Kaplan-Meier analysis. The age of median survival—the age at which 50% of carriers had experienced a cardiac event—at first detection/publication and at last follow-up for all three founder mutations was compared using a log-rank test. Gender differences were calculated, using Fisher exact test. A p-value < 0.05 (2-sided) was considered statistically significant.

In the IVF group, a strong effect of gender on mortality was known from the first publication. In this cohort, we therefore looked at possible associations of gender on
mortality in the IVF cohort at last follow-up in 2012. The magnitude of significant associations was calculated using Cox regression. Hazard ratios (HR) and 95% confidence interval (CI) were calculated.

RESULTS

IVF families
In 2008, haplotype sharing analyses revealed a risk haplotype harboring the DPP6 gene on chromosome 7q36 in ten families with IVF. In these severely affected IVF-risk haplotype carriers (n=60 carriers, 10 families), the median age of survival was 44.6 years (95%-CI: 36.8-52.4 years) in 2008 and increased to 68.2 years (95%-CI: 64.6-71.7 years; p<0.001) in 2012 when new families were added to our analyses (n=235 carriers, 20 families, Table 1, Figure 1a). Figure 1b shows the step by step increase in the median age of survival over time, due to the inclusion of more mutation carriers over the years. Although gender distribution was different between 2008 and 2012 (61.7% males in 2008 vs. 50.6% in 2012), this difference was not significant (p=0.15).

In 2012, the median age of survival in males was 59.9 years compared with 82.4 years in females (p<0.001), with a hazard ratio of 3.69 (95% CI 2.28-5.95, Figure 1c). In 2012, the median age of survival of all carriers was 68.2 years (including the probands) compared with 70.0 years of the carriers without the 20 probands (p=0.28).

SCN5A-overlap syndrome family
In 1999, linkage analyses in a large pedigree with a long QT and Brugada overlap syndrome revealed linkage to SCN5A. After DNA sequencing, the p.1795insAsp mutation syndrome was detected in 86 carriers and obligate carriers. In the subsequent 10

<p>| Table 1. Median age of (50%) survival in three inherited cardiac diseases at first and last analysis |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>First analysis</th>
<th>Last analysis</th>
<th>Year</th>
<th>mutation carriers (n)</th>
<th>Males (%)</th>
<th>Median survival (95% CI)</th>
<th>Year</th>
<th>mutation carriers (n)</th>
<th>Males (%)</th>
<th>Median survival (95% CI)</th>
<th>p-value (median survival)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>2008</td>
<td>60</td>
<td>37 (61.7%)</td>
<td>44.6 years (36.8-62.4)</td>
<td>2012</td>
<td>235</td>
<td>119 (50.6%)</td>
<td>68.2 years (64.6-71.7)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ventricular</td>
<td>2009</td>
<td>197</td>
<td>95 (48.2%)</td>
<td>69.7 years (61.3-78.2)</td>
<td>0.049</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibrillation (IVF)</td>
<td>2012</td>
<td>370</td>
<td>172 (46.5 %)</td>
<td>65.2 years (62.0-68.3)</td>
<td>0.046</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SCN5A overlap syndrome (SCN5A-gene)</td>
<td>1999</td>
<td>86</td>
<td>44 (51.2%)</td>
<td>56.1 years (48.0-64.2)</td>
<td>2009</td>
<td>197</td>
<td>95 (48.2%)</td>
<td>69.7 years (61.3-78.2)</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2010</td>
<td>89</td>
<td>48 (54.0%)</td>
<td>63.5 years (59.1-68.0)</td>
<td>2012</td>
<td>370</td>
<td>172 (46.5 %)</td>
<td>65.2 years (62.0-68.3)</td>
<td>0.046</td>
<td></td>
</tr>
</tbody>
</table>
years, genetic cascade screening of relatives in this extended family identified 109 additional (including obligate and putative) mutation carriers. The median age of survival increased from 56.1 years in 1999 (n=86 carriers, 95%-CI: 48.0-64.2 years) to 69.7 years in 2009 (n=197 carriers, 95%-CI: 61.3-78.2 years; p=0.049) (Figure 2). Gender distribution was not different between the cohorts of 1999 and 2009 (51.2% males vs. 48.2% males; p= 0.70).

**Arrhythmogenic cardiomyopathy (ACM) families**

More recently, in 2010, a cohort of unrelated index patients with ACM (either dilated cardiomyopathy (DCM) or arrhythmogenic right ventricular cardiomyopathy (ARVC)) was analyzed for mutations in the PLN gene. A 3 bp deletion (c.40_42delAGA; p.R14del) in the PLN gene was identified in 15% of the DCM- and 12% of the ARVC-index patients. The median age of survival increased from 63.5 years in 2010 (n=89 carriers from 34 families), 95%-CI: 59.1-68.0 years) to 65.2 years in 2012 (n= 370 carriers from 83 families), 95%-CI: 62.0-68.3 years; p= 0.046) (Figure 3). Gender distribution was not different between the cohorts of 2010 and 2012 (54.0% males vs. 46.5% males; p= 0.24).

**Figure 1a.** Survival in carriers of a risk haplotype for idiopathic ventricular fibrillation (IVF) in 2008 and 2012.

**Figure 1c.** Survival in carriers of a risk haplotype for idiopathic ventricular fibrillation (IVF) in males and females in 2012.
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**Figure 1b.** Median age of (50%) survival between 2008 and 2012 in carriers of a risk haplotype for idiopathic ventricular fibrillation (IVF).

**Figure 2.** Survival in carriers of the SCN5A overlap syndrome in 1999 and 2009.

**Figure 3.** Survival in carriers of an arrhythmogenic cardiomyopathy in 2010 and 2012.
DISCUSSION

This study reveals that the median age of survival in three different genetically-mediated cardiac diseases significantly increased once cascade screening and testing of new families began in the years following the sentinel disease-gene discovery publication. Our most extensively studied cardiac disease, with the most pronounced difference in median age of survival, is IVF. The median age of survival gradually increased by nearly a quarter century in just 4 years. In 2008, at our department, families with a distinct severe phenotype of ventricular fibrillation or sudden cardiac death without a cause were selected for research purposes. The pedigrees of these families consisted of index cases with often multiple family members with sudden cardiac death. In these families, the risk haplotype was identified. In the following years, new (alive and asymptomatic) family members were tested and new families with the risk haplotype were identified. Figure 1b shows the gradual increase in the median age of survival over time in IVF, due to the inclusion of more mutation carriers over the years. It also clearly demonstrates that during the process of testing relatives and discovering new families with the risk haplotype, inclusion of new families (evaluated because of cardiac events) led to a small decrease in the median age of survival. Subsequently, after testing ‘healthy’ relatives, the median age of survival increased. After a certain number of carriers was identified, (n=168, figure 1b), a plateau was reached and the inclusion of new individuals and new families no longer influenced the median age of survival, suggesting that the median age of survival at the plateau most likely represents the ‘true’ mortality risk for this genetic disease. This genetic heart disease quantitatively illuminates just how large the effect of ascertainment bias can be on the median age of survival over time, due to the selection of severely affected families with many cases of VF or SCD at young age at the start of the study, followed by the gradual inclusion of more asymptomatic carriers. In IVF, survival was significantly different between male and female carriers. The effect of the ascertainment bias in this disease is therefore also seen in the gender distribution in the publication cohort from 2008, in which more males are included, while you would expect an equal ratio of males to females in an unselected population of carriers of an autosomal dominantly inherited disease. It is important to acknowledge that in inherited diseases where the expression of the disease is different between males and females, the ascertainment bias can also be shown by an unequal gender distribution. Furthermore, we looked at the effect of the probands on the age of survival. Exclusion of the probands (since these are in general the most severely affected cases) in our analyses did not change the results, indicating that this is not influencing the difference in age of
survival over time.
In the large SCN5A overlap syndrome family, the median age of survival increased by more than a decade. In this group, the ratio of males and females was not statistically different at the start of the study compared with the end of the study. This family originally came to our attention because of the specific phenotype and the high incidence of nocturnal sudden death. Patients are characterized by bradycardia-dependent QT prolongation, sinus node dysfunction and conduction abnormalities. Pacemaker implantation is believed to be effective in preventing sudden death.\textsuperscript{9} We therefore censored all living carriers at the moment of DNA diagnostics or pacemaker implantation (if patients died before DNA diagnostics was performed (obligate carriers) or when they received a pacemaker, based on their phenotype, before DNA diagnostics was performed). This moment of censoring resulted in relatively less patient-years (follow-up starting at birth) of newly identified carriers in the 2009 analysis compared to the first analysis and added relatively few events to the survival analysis. Despite this, the median age of survival still increased by over 13 years.

Among our most recently discovered founder mutation, PLN-mediated ACM, the median age of survival has already increased by 2 years within 2 years of its discovery. The ratio of males and females was not statistically different. Patients can have an ACM with abnormalities of the left and right ventricle. Treatment consists of medication in case of arrhythmias or heart failure, and ICD implantation and/or cardiac transplantation in the most severe cases. The relatively small increase of the median age of survival can be due to the short follow-up period. It can also suggest less ascertainment bias at the beginning of the study; a large group of dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) index patients was selected, purely on their phenotype and not so much on their family history of sudden cardiac death. Since we did not take into account the treatment of our patients, the treatment of carriers could have positively influenced the median age of survival at the end of our study.

The inclusion of alive and asymptomatic patients, who are able to come to our department to perform DNA diagnostics, involves a bias towards living years. However, also first degree relatives with sudden cardiac death at a young age were included in our analyses in all three inherited diseases.

To our knowledge, we hereby clearly, and for the first time in a direct comparison, elucidate and quantify the effect of ascertainment bias on the prognosis in large groups of patients with an inherited cardiac disease. Clearly, this could have a significant impact on the information physicians give to their patients and on the treatment choices made in these families (e.g. pacemaker or ICD implantation). Our results might serve as a
proof-of-principle for all inherited diseases. They suggest an overestimation of disease severity, especially in first publications. It is therefore important that clinical follow-up data of larger cohorts will be collected and published for inherited diseases to enable physicians to give patients more accurate information on their disease prognosis.

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