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LIFETIME PROBABILITIES OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE US

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Abstract

Health care policies regarding hematopoietic stem cell transplantation (HCT) must address the need for the procedure as well as the availability of stem cell sources: bone marrow, peripheral blood, or umbilical cord blood (UCB). However, data with respect to the lifetime probability of undergoing HCT are lacking.

This study was undertaken to estimate the latter probability in the United States (US), depending on age, gender and race. We used data from the Center for International Blood and Marrow Transplant Research, the US Surveillance, Epidemiology and End Results Program and the US Census Bureau and calculated probabilities as cumulative incidences. Several scenarios were considered: assuming current indications for autologous and allogeneic HCT, assuming universal donor availability, and assuming broadening of HCT use in hematologic malignancies.

Incidences of diseases treated with HCT and of HCTs performed increase with age, rising strongly after age 40. Among individuals older than 40, incidences are higher for men than for women. The lifetime probabilities of undergoing HCT range from 0.23% to 0.98% under the various scenarios.

We conclude that, given current indications, the lifetime probability of undergoing autologous or allogeneic HCT is much higher than previously reported by others and could rise even higher with increases in donor availability and HCT applicability.

INTRODUCTION

Hematopoietic stem cell transplantation (HCT) is an effective therapy for many life-threatening malignant and non-malignant diseases. Depending on the situation, a patient's own (autologous) cells or (allogeneic) cells from a donor are used. Presently, cells for HCT can be collected from bone marrow, peripheral blood, or umbilical cord blood (UCB) [reviewed in [1–4]].

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In planning US health care policies, especially with regard to allocating resources for donor registries and UCB banking, estimates of the probability that one will need an HCT during one's life are critical, but data regarding this probability are lacking.

The objective of this study was to calculate the lifetime probability of undergoing HCT in the United States (US) under various scenarios and its dependence on age, gender and race. The calculations in this study are pertinent to all sources of hematopoietic stem cells for transplantation.

MATERIALS AND METHODS

Data Sources

HCT data were obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR) for patients up to age 70 years (generally the maximum transplant age), who received an HCT for any indication in the US in 2001–2003. The organization of the CIBMTR and its methods for data collection and management are described elsewhere [5].

Because reporting transplants to CIBMTR is voluntary, the database does not include all HCTs performed. Currently there is no US database that includes all allogeneic or autologous HCTs. Based on data available from the National Marrow Donor Program (NMDP) which collects data on most (>90%) unrelated donor transplants in the US, the Bone Marrow Transplant Information Network (BMT Infonet) which attempts to survey all US transplant centers yearly, and the U.S. Hospital Discharge Database from the Health Cost Utilization Project (HCUP), we estimate that in the above mentioned years the CIBMTR collected transplant data on about 55% of autologous and 50% of allogeneic HCTs performed in the US. Therefore, CIBMTR autologous and allogeneic HCT numbers were multiplied by 1.82 and 2, respectively, to estimate total numbers of HCTs in the U.S. These adjustment factors were applied uniformly to all subgroups of patients reported to the CIBMTR and described in Table 1, assuming that the cases reported to the CIBMTR are a random sample of all HCTs performed in the US. This assumption appears to be justified by comparison with data collected by the organizations listed above. The distribution of diseases and transplant types is also similar to the distribution in the Europe-wide survey of transplant activity conducted yearly by the European Group for Blood and Marrow Transplantation (EBMT) [6].

The incidences of malignancies commonly treated with HCT were obtained from the Surveillance, Epidemiology and End Results (SEER) Program of the US National Cancer Institute [7]. SEER data are derived from registries covering approximately 26% of the US population and do not include non-malignant HCT indications [7]. We used the SEER 13 database, which contains cases diagnosed from 1992 to 2002 [7]. Using publicly available software (SEER*Stat, version 6.1.4), we calculated incidence rates per 100,000 persons up to age 70 for the years 2000–2002. Considering children and adults separately (see Table 1), only diagnoses that accounted for 5% or more of the HCTs in the CIBMTR database were included; diseases for which transplants are rarely done were not considered. Disease incidences were also calculated separately by age decades as described in Table 2.

Scenarios

We calculated the probability of undergoing HCT, for people in the US, by age 70 years, under 4 different scenarios (* = under current indications):

1. when the HCT is autologous *;
2. when the HCT is allogeneic with universal donor availability *; For this scenario it was assumed that there would be no restriction in the availability of an HLA-identical

sibling (or comparable) donor. Since in reality only 30% of allograft candidates have an HLA-identical sibling [8, 9], the actual number of such transplants was multiplied by 3, to estimate the "unrestricted" number of allogeneic HCTs in a setting of universal donor availability. Some patients without an HLA-identical sibling currently receive allogeneic transplants from alternative donors; we did not include those numbers in this calculation.

3. when the HCT is either autologous* or allogeneic * with universal donor availability; For this scenario, the HCT numbers under Scenarios 1) and 2) are combined. In arriving at these combined numbers, we assumed that patients currently receiving autologous transplants for diseases where allotransplants are generally preferred would receive an allotransplant if a donor were available, such as transplantation for leukemia. Consequently, numbers of autotransplants for acute and chronic leukemia included in Scenario 1 were not included in the numbers for Scenario 3 since they were already counted under Scenario 2 (in the multiplication by 3, assuming universal donor availability);

4. when the HCT is either autologous or allogeneic AND there is universal donor availability AND current indications are expanded so that 50% of the patients with cancers treatable with HCT receive a transplant. In planning this scenario, we compared the incidence of the cancers treatable with HCT with the estimated annual number of HCTs performed. This indicated that 15–20% of adults younger than 70 years with these cancers received HCT. This varied according to specific indication. For example the proportion of patients with leukemia receiving HCT was 10–15%. The proportion of children with neuroblastoma receiving HCT was about 35% and the proportion of adults (younger than 70) with multiple myeloma receiving HCT was 40–45%. Considering these percentages, we took 50% as an "upper limit" for Scenario 4 and calculated the numbers of HCTs that would be performed if half of the patients with one of *all* the diseases treatable with HCT would receive a transplant. The "upper limit" was not set at 100%, since under any envisioned circumstance a considerable number of patients would receive therapies other than HCT for a variety of reasons including having low risk disease or highly refractory disease, co-morbidities or socio-economic factors.

Statistical Analysis

First, the average annual incidences of HCT per 100,000 people were computed by age decade under each scenario. Incidences of SEER diagnoses per 100,000 people were normalized to average mid-year US population between the years 2001–2003 [10]. Next, the probability of receiving an HCT was calculated as a cumulative incidence under each scenario, using the cumulative incidence estimator with death in the absence of HCT as a competing risk [11, 12] To calculate the cumulative incidences, the proportion of individuals at a particular age who are mathematically "at risk" to undergo an HCT (i.e. the proportion of individuals at that age who are alive without an HCT), was approximated by the overall probability of being alive at that age, utilizing data from the US life tables for 2002 [13]. It is not possible to obtain the actual proportion of individuals in the overall population at risk at a given age; however, since the proportion of patients actually receiving transplantation or being diagnosed with a transplantable disease is very small, the proportion at risk is quite close to the proportion of individuals alive at a given age. The yearly increment in the cumulative incidence estimate is the proportion of individuals alive at the beginning of the year times the incidence of receiving an HCT in the next year. Although the patient populations reported to the CIBMTR are adjusted for under-reporting, individual patient-level data is not needed to perform this calculation. Since these calculations are performed on group-level data, they are not available in standard software packages. A SAS/IML program was written to perform the cumulative incidence

calculations. Probabilities were also calculated for subgroups of patients defined by gender and race. Race classifications for HCT recipients and for the population of patients with hematological malignancies were made by CIBMTR and SEER respectively. Due to differences in classification by CIBMTR and SEER databases, racial subgroup analysis for incidences and lifetime probabilities was limited to Caucasians and African-Americans.

RESULTS

Numbers of transplants under Scenarios 1–4

Table 1 depicts the average annual total of US HCTs under each scenario and the distribution of HCTs by gender, race and transplant indication (plus the respective percentages of the total). A comparison of the numbers of autologous HCTs (Scenario 1) and allogeneic HCTs (Scenario 2) shows that the latter would exceed the former, if donor limitations did not exist. As expected, the highest HCT numbers are found when current indications are expanded (Scenario 4).

Distribution by gender is similar under all 4 scenarios, with more male than female HCT recipients. The distribution by race is also similar under all 4 scenarios. For autologous HCT, the two most common indications in children are neuroblastoma and central nervous system tumors, while in adults they are multiple myeloma and lymphoma. For allogeneic HCT (Scenario 2) and for HCT in general (Scenario 3) leukemia is the most common indication in both age groups.

When current indications are expanded to include a larger proportion of individuals with malignancies considered treatable by HCT (Scenario 4), the most common cancers treatable with HCT are leukemia for children and lymphoma for adults.

Incidences of HCT, by age decade

Table 3 shows the incidences of HCT by age. Incidences are considerably higher in the 5^{th}–7^{th} decades than in the 1^{st}–3^{rd} decades of life, under all 4 scenarios. This “age effect” is observed for men and women of both racial groups. Table 3 shows that the higher transplant numbers for men versus women in Table 1 derive mainly from gender differences in disease incidences in the 5^{th}–7^{th} age decades.

Cumulative probabilities of receiving an HCT, by age decade

Fig.1 shows that when the incidence rates in Table 3 are used to calculate cumulative probabilities by age, there is a sharp increase in probability of HCT after age 40. Presently, for an average person in the US, the lifetime probabilities of receiving an HCT are 0.23%, 0.25%, 0.46%, or 0.98%, under Scenarios 1, 2, 3 and 4, respectively. Table 4 shows that when the cumulative probabilities of Fig.1 are stratified by gender and race, the differences between the incidences for men and women, as shown in Table 3 for the 5^{th}–7^{th} age decade, translate into comparable differences between cumulative probabilities in Table 4 (with the exception of African-American men and women under Scenario 1).

It should be noted that in Table 3 and Table 4, in a few cases, the HCT incidences, and the corresponding cumulative probabilities, for the total population are higher than for each subgroup. This is due to the higher application of HCT among individuals who are not identified as Caucasian or African-American.

DISCUSSION

Whether autologous or allogeneic stem cells are used for an HCT depends on the underlying disease and the planned treatment strategy. When an immune anti-cancer effect is wanted, or

when an inherited bone marrow defect in the patient needs correction, or when a cancer-free autologous graft can not be harvested from the patient, an allogeneic transplant becomes the primary choice. Performing an allogeneic HCT depends upon finding a suitable donor, ideally an HLA-identical relative. Such a donor is unfortunately only available for about a third of patients in the US [8, 9]. The next best option is an HLA-identical or minimally HLA-mismatched unrelated donor transplant, using cells collected from a healthy adult volunteer donor or previously collected and stored UCB cells made available for public use. In some centers UCB transplantation is now the preferred choice for unrelated donor HCT in children who do not have an HLA-identical related donor [14]. During the years selected for this study, about 25% of allogeneic HCTs performed used adult or cord blood unrelated donors [CIBMTR data]. Despite these alternative sources of grafts, more than half of patients in need of transplantation still do not have an available donor.

The average annual number of HCTs, either autologous or allogeneic, which we calculated for the US (about 17,000 under Scenario 3, assuming universal donor availability, Table 1), is similar or higher than the average annual number of other generally accepted medical procedures in the US, e.g., kidney transplantation, with an average of about 15,000 per year [15] and surgery for cleft palate / cleft lip, with an average of about 5,000 per year [16].

When calculating the lifetime probabilities, several assumptions were made that deserve discussion. Under Scenario 1, CIBMTR data indicate that the lifetime probability of undergoing an autologous HCT in the US is about 1:400 if the indications for autologous HCT do not change much during the next 70 years. This, of course, may not be true. Advances in HCT technology may lead to its use for new indications, new pharmaceutical developments may replace HCT for some diseases, or both may occur; the effect of these advancements could increase, decrease or leave unchanged the lifetime probabilities estimated in this study. Under Scenarios 2 and 3, the lifetime probabilities are 1:400 and 1:200, respectively, for undergoing allogeneic HCT or either autologous or allogeneic HCT. Those probabilities are partly speculative, because of the assumption of universal donor availability. However, these probabilities may be realized with increased numbers of donors and/or UCB units and/or strategies to accommodate greater degrees of donor-recipient HLA disparity. A bank with sufficient allogeneic UCB units could provide suitable transplants for most US patients in need, because of the possibility of using UCBs with 1 or 2 HLA mismatches [17], and when strategies become available to overcome limitations of low cell numbers [18–22]. The size required for such a donor bank is discussed elsewhere [23] and must take into account differences, if any, in outcome with varying degrees of HLA matching and varying cell doses [24].

Under Scenario 4, the calculated probability of almost 1:100 is based on the speculation that many changes in current practice will enable more widespread use of HCT in the future in patients with diseases where efficacy has already been demonstrated. A comparison of the probabilities under Scenarios 3 and 4 shows how much a change in scenario can affect the lifetime probability of undergoing an HCT. At the moment about 17% of patients who are diagnosed with diseases potentially treatable with HCT actually undergo HCT (as outlined in the Materials and Methods section), in contrast to the 50% we empirically selected as an “upper limit”. It is unlikely that the percentage would be higher than this since patients may not require transplantation, may be treated with other therapies, may have comorbidities that would preclude transplantation or may have socio-economic barriers to transplantation. The usage of HCT is limited by consideration of the risk to benefit ratio of this therapy, which carries significant treatment-related mortality, versus other (less aggressive) therapies [4]. Major improvements in safety and efficacy of HCT are required to realize Scenario 4. It is of interest that during the study period, we estimated that 40–45% of patients diagnosed with multiple myeloma up to age 70 years received HCT (>95% autologous HCT). During this time, there was general consensus that autotransplant was the preferred therapy (though more recent

studies have brought this into question) and that the procedure could be safely done even in older patients.

Regardless of scenario or transplant practice, yearly HCT rates would increase if uninsured Americans, which included 11% of children and 15% of non-elderly adults in 2003 [25], had full access to health care. Unequal access to health care may account for some of the discrepancy between the proportion of HCTs received by African-Americans (9%, Table 1, Scenario 3) and their representation of about 13% in the US population [26].

As stated in the Materials and Methods section, our adjustment for under-reporting assumed that transplants reported to the CIBMTR are a simple random sample of patients receiving transplant. There may be inherent differences in the types of patients treated by centers reporting versus not reporting to the CIBMTR, which would result in a biased adjustment for under-reporting. However, inspection of data reported to NMDP, BMTInfoNet and the EBMT, suggest that CIBMTR is representative. A similar bias could occur from the use of group-level data from SEER.

In conclusion, whatever the future developments in HCT practice, our results show that the lifetime probability of undergoing HCT is much higher than the probabilities previously reported by others [27–29], which ranged from 1:2700 to 1:200,000. These results are important for planning donor registries, UCB banks and health insurance policies.

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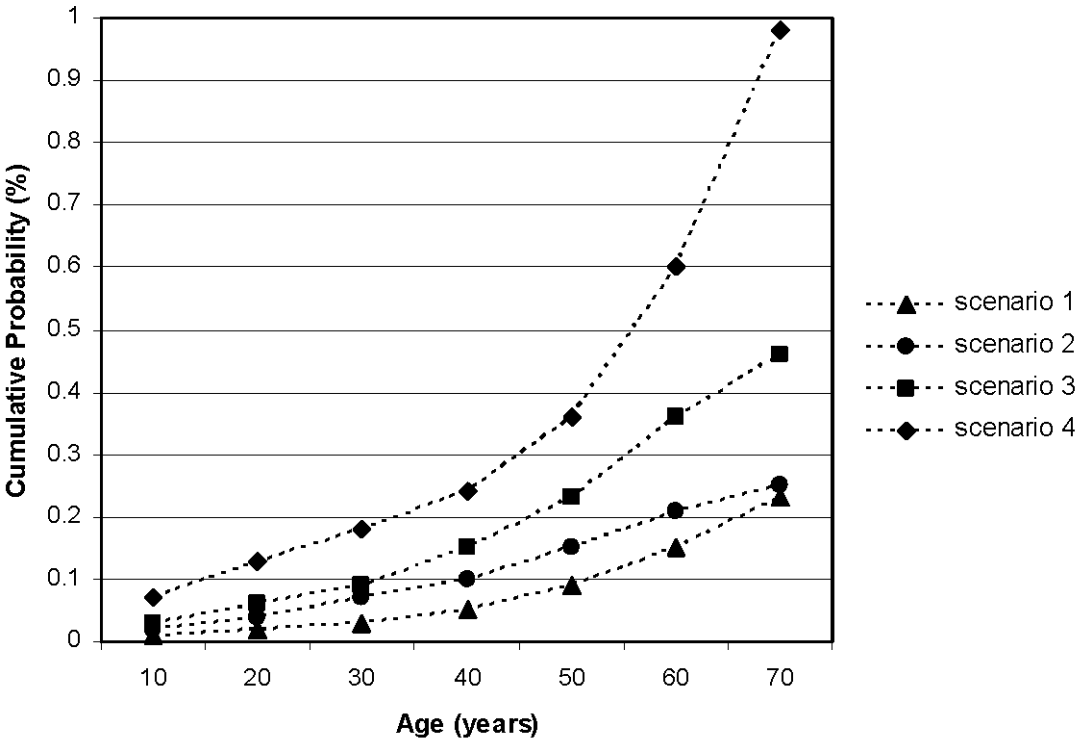

Fig. 1. Cumulative probabilities that an HCT has been received by a specific age, under 4 scenarios

For a description of the 4 scenarios, see the Materials and Methods section. The values under Scenario 3 do not always equal the total of the values under Scenario 1 plus Scenario 2. For an explanation, see the Materials and Methods section.

Table 1

Average annual HCT numbers, under 4 scenarios [a, b]

	Scenario 1	(%)	Scenario 2	(%)	Scenario 3	(%)	Scenario 4	(%)
Total number of HCTs	7,811	(100)	9,480	(100)	16,796	(100)	32,484	(100)
Male recipients	4,434	(57)	5,644	(60)	9,826	(59)	18,619	(57)
Race								
Caucasian	6,310	(81)	7,192	(76)	13,104	(78)	27,215	(84)
African American	831	(11)	750	(8)	1,532	(9)	3,765	(12)
Other	670	(9)	1,538	(16)	2,160	(13)	1,504	(5)
Cases for ages <20 years	641	(100)	1,716	(100)	2,335	(100)	4,972	(100)
Leukemia	22	(3)	1,060	(62)	1,060	(45)	1,748	(35)
Non-Hodgkin Lymphoma	47	(7)	64	(4)	111	(5)	464	(9)
Hodgkin Disease	92	(14)	10	(1)	102	(4)	447	(9)
Multiple Myeloma	0	(0)	0	(0)	0	(0)		
Neuroblastoma	235	(37)	6	(0)	241	(10)	310	(6)
CNS Tumors	125	(20)	0	(0)	125	(5)	1,173	(24)
Sarcoma	64	(10)	0	(0)	64	(3)	830	(17)
Myelodysplasia	1	(0)	42	(2)	42	(2)		
Aplastic Anemia	0	(0)	174	(10)	174	(7)		
Other Malignancies[c]	51	(8)	6	(0)	57	(2)		
Other non-malignant[d]	5	(1)	354	(21)	359	(15)		
Cases for ages 20–70 years	7,170	(100)	7,764	(100)	14,461	(100)	27,512	(100)
Leukemia	459	(6)	4,294	(55)	4,294	(30)	7,390	(27)
Non-Hodgkin Lymphoma	2,330	(32)	1,436	(18)	3,766	(26)	13,626	(50)
Hodgkin Disease	858	(12)	106	(1)	964	(7)	2,850	(10)
Multiple Myeloma	2,961	(41)	322	(4)	3,284	(23)	3,646	(13)
Neuroblastoma	3	(0)	0	(0)	3	(0)		
CNS Tumors	21	(0)	2	(0)	23	(0)		
Sarcoma	33	(0)	8	(0)	41	(0)		
Myelodysplasia	15	(0)	956	(12)	956	(7)		
Aplastic Anemia	0	(0)	204	(3)	204	(1)		
Other Malignancies[c]	427	(6)	370	(5)	797	(6)		
Other non-malignant[d]	63	(1)	66	(1)	129	(1)		

[a] For a description of the 4 scenarios, see the Materials and Methods section.

[b] The numbers under Scenario 3 do not always equal the total of Scenario 1 plus Scenario 2. For an explanation, see the Materials and Methods section.

[c] Other Malignancies: breast cancer, ovarian cancer, germ cell tumors, renal cell carcinoma, lung cancer, hepato-biliary cancer, pancreatic cancer, cervical cancer, colorectal malignancies, small cell lung cancer, prostate cancer, melanoma, other not specified or missing diagnosis.

[d] Other non-malignant diseases: sickle cell anemia, thalassemia, Fanconi anemia, Diamond Blackfan Anemia, Glanzmann thromboasthenia, congenital amegakaryocytic thrombocytopenia, other congenital platelet abnormalities not otherwise specified, autoimmune disorders, Severe combined immunodeficiency syndromes, other diagnosis (not reported). Data on non-malignant diseases are not available through SEER.

Abbreviations: CNS, central nervous system.

Table 2

Incidence per 100,000 of malignant diseases most commonly treated with hematopoietic stem cell transplantation. SEER database from 2000 to 2002.

Age	**0 – 9**	**10 – 19**	**20 – 29**	**30 – 39**	**40 – 49**	**50 – 59**	**60 – 70**
Diseases							
Leukemia	5.8	2.9	2.3	3.4	5.5	12.0	28.0
Non-Hodgkin's lymphoma	0.7	1.6	2.7	5.7	12	25.0	47.1
Hodgkins Disease	0.2	1.9	4.1	3.5	2.7	2.2	3.1
Multiple Myeloma	-	-	0.06	0.5	2.0	7.6	17.2
Neuroblastoma[1]	1.5	0.1	-	-	-	-	-
CNS tumors[1]	3.5	2.3	-	-	-	-	-
Sarcomas[1]	1.3	2.8	-	-	-	-	-

[1] CNS tumors and sarcomas are prevalent in patients older than 19 years however it is not a common indication for hematopoietic stem cell transplantation in older patients and thus not included in the table after this age; neuroblastoma is uncommon in patients older that 19 years.

Abbreviations: CNS, central nervous system.

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Table 3

Average annual incidences[a] of HCT per age decade, by gender and race[b], under 4 scenarios[c, d].

Age decades	**0–10**	**10–20**	**20–30**	**30–40**	**40–50**	**50–60**	**60–70**
Category							
Scenario 1							
Total population	**1.0**	**0.6**	**1.4**	**2.0**	**3.4**	**7.2**	**8.6**
Caucasian Men	1.0	0.7	1.5	2.2	3.7	8.1	11.1
Caucasian Women	0.8	0.5	1.2	1.7	3.0	6.0	6.2
African American Men	0.8	0.4	1.0	1.7	3.4	7.6	8.8
African American Women	0.9	0.4	1.2	2.0	3.2	6.8	6.5
Scenario 2							
Total population	**2.2**	**2.1**	**2.1**	**3.1**	**5.5**	**6.8**	**4.3**
Caucasian Men	2.0	1.8	1.9	3.2	6.2	8.8	6.2
Caucasian Women	1.6	1.3	1.4	2.4	4.6	4.9	2.8
African American Men	1.7	1.9	1.4	2.5	3.2	5.7	2.0
African American Women	1.5	1.3	1.3	2.0	2.5	3.7	1.2
Scenario 3							
Total population	**3.1**	**2.6**	**3.3**	**4.9**	**8.7**	**13.6**	**12.4**
Caucasian Men	3.0	2.5	3.3	5.2	9.7	16.5	16.9
Caucasian Women	2.4	1.7	2.5	3.9	7.3	10.4	8.7
African American Men	2.5	2.3	2.3	4.1	6.4	12.8	10.2
African American Women	2.4	1.7	2.4	3.7	5.6	10.3	7.5
Scenario 4							
Total population	**6.6**	**5.8**	**4.6**	**6.6**	**11.2**	**23.4**	**47.7**
Caucasian Men	7.8	5.2	5.2	8.3	14.6	30.5	62.9
Caucasian Women	6.7	5.5	4.5	5.7	9.0	19.5	42.2
African American Men	4.7	4.2	4.9	10.2	16.9	30.6	60.4
African American Women	3.9	4.2	5.0	6.1	9.5	21.4	37.6

[a] Cases/100,000 individuals.

[b] Incidences could only be calculated for Caucasians and African Americans (see the Materials and Methods section).

[c] For a description of the 4 scenarios, see the Materials and Methods section.

[d] The numbers under Scenario 3 do not always equal the total of Scenario 1 plus Scenario 2. For an explanation, see the Materials and Methods section.

Table 4

Cumulative probabilities that an HCT has been received by a specific age, by gender and race[a], under 4 scenarios[b, c].

Age (years) Category	10	20	30	40	50	60	70
Scenario 1							
Total population	**0.01%**	**0.02%**	**0.03%**	**0.05%**	**0.09%**	**0.15%**	**0.23%**
Caucasian Men	0.01%	0.02%	0.03%	0.06%	0.10%	0.17%	0.26%
Caucasian Women	0.01%	0.01%	0.03%	0.04%	0.08%	0.13%	0.19%
African American Men	0.01%	0.01%	0.02%	0.04%	0.08%	0.14%	0.19%
African American Women	0.01%	0.01%	0.03%	0.05%	0.08%	0.14%	0.19%
Scenario 2							
Total population	**0.02%**	**0.04%**	**0.07%**	**0.10%**	**0.15%**	**0.21%**	**0.25%**
Caucasian Men	0.02%	0.04%	0.06%	0.09%	0.15%	0.23%	0.28%
Caucasian Women	0.02%	0.03%	0.05%	0.07%	0.12%	0.16%	0.18%
African American Men	0.02%	0.04%	0.05%	0.08%	0.11%	0.15%	0.16%
African American Women	0.02%	0.03%	0.04%	0.06%	0.09%	0.12%	0.13%
Scenario 3							
Total population	**0.03%**	**0.06%**	**0.09%**	**0.15%**	**0.23%**	**0.36%**	**0.46%**
Caucasian Men	0.03%	0.06%	0.09%	0.15%	0.24%	0.39%	0.52%
Caucasian Women	0.03%	0.04%	0.07%	0.11%	0.19%	0.28%	0.36%
African American Men	0.03%	0.05%	0.07%	0.11%	0.18%	0.28%	0.34%
African American Women	0.03%	0.04%	0.07%	0.10%	0.16%	0.25%	0.31%
Scenario 4							
Total population	**0.07%**	**0.13%**	**0.18%**	**0.24%**	**0.36%**	**0.60%**	**0.98%**
Caucasian Men	0.09%	0.16%	0.21%	0.30%	0.43%	0.61%	1.10%
Caucasian Women	0.07%	0.13%	0.17%	0.23%	0.33%	0.53%	0.90%
African American Men	0.05%	0.09%	0.15%	0.25%	0.41%	0.68%	1.07%
African American Women	0.04%	0.09%	0.14%	0.20%	0.30%	0.50%	0.79%

[a] Cumulative probabilities could only be calculated for Caucasians and African Americans (see the Materials and Methods section).

[b] For a description of the 4 scenarios, see the Materials and Methods section.

[c] The numbers under Scenario 3 are not always equal the total of Scenario 1 plus Scenario 2. For an explanation, see the Materials and Methods section.